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TRANSACTIONS OF THE SOCIETY.

PRESIDENTIAL ADDRESS.

I.—THE CENTENARY OF THE ROYAL MICROSCOPICAL SOCIETY 1839–1939. 90. 902

SOME PERSONAL RECOLLECTIONS AND IMPRESSIONS.

By J. E. Barnard, F.R.S.

The Royal Microscopical Society was to have celebrated its centenary in the autumn of 1939 but the outbreak of war necessitated a change of plans. All special meetings and social functions were postponed to some more happy time, and addresses which were to have been given took the form of papers contributed during 1940 to the Journal of the Society. The Journal for that year thus assumed much of the character of a centenary volume, in which the presidential address was to have been included ; but a prolonged period of ill health has caused delays which, to my profound regret, have prevented publication of the address until now.

The Royal Microscopical Society was founded in 1839, under the title of The Microscopical Society of London. It is difficult for us of the present day to recapture the atmosphere of a hundred years ago. British makers had already attained leadership in microscope design, largely owing to the help and exacting demands of the amateur microscopist, but, judged by present-day standards, instruments were still primitive, technique was crude, and the field of knowledge was narrowly circumscribed. The article on "Optics" in the *London Encyclopœdia,* published in 1829, contains this illuminating remark :

"Microscopes, though but toys compared with telescopes, nevertheless deserve to be rendered as perfect as possible ; for they yield not to them

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in quantity and variety of rational amusement which they are capable of introducing to us (though not of the sublime description of the wonders of the heavens). Compound microscopes, though not so much to be depended upon for the purposes of discovery and philosophical investigation as single lenses, are still best adapted for recreation." (Quoted from "Origin and Development of the Microscope," Disney, Hill and Baker (1928).)*

However, the tide had already started to flow. By about 1840, the date around which our interest is centred at the present moment, serious workers were beginning to use the microscope intensively in the examination of tissue structure, and a flood of startling new discoveries had set in. In 1831 Schleiden had discovered that plants are essentially cellular in nature, and in 1838 Schwann had reported that animals too are similarly constituted. Schwann's words are: "There is a principle of development common to most different elementary parts of the organism, viz. cellular formation." (Quoted from "History of Pathology," R. E. Long (1928). Henceforth the cell was to take its proper place in biology. Humoral theories were being discredited and discarded. At this period, then, that is, just a hundred years ago, there was a lively and growing enthusiasm for the microscope and its revelations. Wealthy amateurs and professional men of science were alike attracted: some on account of the novelty and beauty of the objects revealed, and some for deeper reasons. The time was ripe for the foundation of our Society, and it came into being under the earlier title mentioned

> "for the promotion of microscopical investigation and for the introduction and improvement of the microscope as a scientific instrument."

With what unremitting devotion to the achievement of these objects the members have ever since applied themselves the volumes of the Society's Journal eloquently testify.

Of the first fifty years of the Society's activity I propose to say little, for we are particularly fortunate in that in January, 1895, Mr. A. D. Michael took this period as the subject of his presidential address. I can add little from personal knowledge to what he said then, and I have preferred to let Michael speak again, and to a wider audience, many of whom may not have ready access to the older volumes of the Journal of the Society. With this object Michael's address is republished, almost in its entirety, as an appendix to the present address. Some references that were of interest only in Michael's day have been omitted, since, in general, their omission does not alter the interest and accuracy of his publication. I am sure that Fellows will agree with me that it is but a fitting tribute to the memory of a distinguished past President to republish what he wrote as far as possible in the words he himself used. His account of the earlier history of the Society obviously

* Published by the Royal Microscopical Society.

involved much laborious investigation, for which credit must and should be given without reservation.*

I remember Michael. He would be about sixty years old at the time his address was given at the Society's rooms in Hanover Square, and the account he gave of the foundation and first half century of the Society's life must have then been verifiable either from personal knowledge or from the testimony of others who had themselves known the chief actors. It so happens that the period subsequent to the date on which Michael gave his address exactly coincides with the time that I have been connected with the Society. I listened to Michael's address, was nominated for the Fellowship at the very next meeting, and was elected in March, 1895. Many of those who had become prominently associated with the activities of the Society were soon to become familiar figures. Some of those whose names appear on the list of officers or members of Council for that year were already known to me, and from them I received much valuable advice and assistance. Mr. Conrad Beck, one of our oldest surviving Fellows, was already on the Council, and at the time of writing is again serving in the same capacity some forty-five years later, a wonderful record for which I can find no parallel. During this long period he has occupied the presidential chair, and, except for some statutory intervals, has always been at the service of the Society in some capacity or other. Michael himself, as I remember him, was a distinguished-looking man, quiet, and rather reserved in manner; an ideal chairman, who conducted the meetings with dignity and unruffled charm.

The chief difference to me between the meetings of forty-five years ago and those of to-day is the absence now of that sociality which forms so marked a feature in my earliest recollections of the Society. The average number attending the meetings was about the same as now, but in these earlier days some at least of the members would bring a microscope. There were, in fact, usually several microscopes, each with its object of interest. This involved bringing a lamp also, as no systematic arrangements were then made for illuminants such as we have now had at all meetings for some years past. Transport, too, was difficult; it was not simply a question of loading all the necessary accessories on to a car and reaching the meeting room without difficulty. Members commonly had to assist each other if a demonstration of unusual interest was in contemplation. Many times have I helped some member to carry a heavy microscope, and many times been helped in my turn. But it was well worth the trouble; we got to know each other and many life friendships were established. The main difficulty was to end

* It is worthy of note that the microscopes specially built by British makers to the order of the first Council in 1840 are still in the Society's possession and may rightly be regarded as the parents of the modern instrument. From then on the contributions of the members to the design and construction of the instrument as we know it to-day, the formulation of the Society's standard specifications for the objective screw thread, eye-pieces and substage equipment, the introduction of the mechanical substage and other developments, are memorialized throughout the world wherever microscopes are used; and progress still continues.

the meetings, as the formal closing by the chairman was by no means final; in fact, to many the real interest was then only beginning. I think the change to greater formality is probably largely due to a change in the character of the Fellowship. Formerly the amateur element was dominant, and they were all enthusiasts—that was the common bond of union. Latterly the professional worker has been in the ascendant, while the amateur has practically disappeared, so that a man whose daily work may be of great interest does not want to prolong it unduly at the end of his working day. It is not so much that his interest is less keen than that of the amateur but his natural inclination is to relax; he does not feel inclined to continue late into the evenings, nor in many cases has he then any surplus energy to expend.

Another change is that the meetings now are tamer; enthusiasms do not overflow, and rarely is the chairman called upon to maintain or to restore order. Perhaps this is all to the good, but, frankly, as one who has occupied the chair frequently enough, I have often sighed for the old days, with their blunt yet transparent honesty. On looking over the programmes of meetings with which I have been in familiar contact, I find that while the subjects discussed have always been varied there were successive periods when particular questions have recurred. This variation of subject has been in part influenced by the interests of the President for the time being. Thus the next President to Michael, with his specialized interests, was E. M. Nelson, who for a long period was an outstanding figure in English optics. Nelson threw himself into what was known as the Abbe controversy with characteristic vigour. There is no need to go over the ground again. Those of us who were interested got endless thrills as the attacks were made and countered. The services of Abbe to microscopical optics, both theory and practice, are too well known and established to be fitting subjects for debate in these days. But there is no doubt that at the time of its first presentation his work did provide scope for much discussion. At this early period some of Abbe's followers were more enthusiastic than their master.

Nelson did outstanding service to practical microscopy. He strove his utmost to get the best possible results from the microscope, and urged others to do likewise and to spare no pains in their efforts. To express the ideal for which he strove Nelson introduced the terms "critical microscopy" and "the critical image," terms which perhaps were never very good and which certainly are now often misused. He insisted on the advisability of using an objective at its greatest practical numerical aperture, in contradistinction to the earlier Abbe pronouncements favouring reduced apertures. Nelson's work was particularly opportune and resulted in a widespread change in technical procedure which even yet has not been fully exploited and of which the rationale is still but partially understood. His contributions to our Journal were numerous, both during the period of his occupation of the presidential chair and afterwards. As an observer he was unrivalled, partly because the technical methods he advocated were designed to get the very best out of his optical equipment, but also because of an exceptional keenness

of vision that he took pains to preserve and develop to the utmost. Thus he was an advocate of the method of working with the microscope in a darkened room, never making observations of importance until his eyes had been rested in partial or complete darkness for some time previously. Those who work habitually at a laboratory bench with full daylight streaming into their eyes have no idea of the difference modification of the general lighting of a room can make to accurate work when difficult questions of interpretation are involved. He left an impression on our Society that will remain for many years to come.

In the year 1901 began a series of discussions on the theory of the formation of the microscopical image that has continued almost to the present day. Some workers were not in agreement with what was known as the *Abbe Diffraction Theory*, with all its implications, but it was left to Mr. J. W. Gordon to launch a frontal attack. This he did at the June meeting of 1901 in a paper which for thoroughness and attention to detail is almost unrivalled in the records of the Society. Many did not accept his contentions, criticism was sometimes harsh and unbridled, but Gordon was a K.C. whose legal experience made him a dangerous exponent of any cause that he upheld. Those were indeed great days, that dwell vividly in my memory. The same subject has been discussed many times since but never, I think, with the same enthusiasm. Great names appear in the course of or as the result of these meetings, Silvanus Thompson, Alfred W. Porter, Lord Rayleigh, and many others. In 1903 another equally lengthy paper by Gordon on "The Helmholtz Theory of the Microscope," and two papers by Lord Rayleigh "On the Theory of Optical Images with Special Reference to the Microscope" followed. It is not my purpose to attempt to appraise the value of these papers, they can be read in the Journal by those interested. They all added to knowledge and clarified discussion on a subject that up till then had not been presented so fully elsewhere. The Microscopical Society became, in fact, a living centre of interest in matters of general optics, as indeed it always should be.

In the same year, 1903, a notable paper appeared by Siedentopf on the ultra-microscope, a none too happy term which has nevertheless persisted and been widely misapplied. The principle of dark-ground illumination had long been known and practised, but the Tyndall effect had not previously been applied to suitable problems in microscopy. It has been of considerable use in the study of colloidal solutions, although it has not proved of such value in the observation of living organisms as the designer of the apparatus apparently anticipated.

In 1905 an Optical Convention was held, an abridged account of which is published in our Journal for that year. The subjects of optical glass and the polishing of glass surfaces were discussed, though it was only at a much later period that these matters became questions of importance in practical optics. The subject of micrometallurgy was also raised by J. E. Stead, F.R.S., and his paper was a notable contribution to the subject.

In 1904 an account was published in the *Zeitschrift für Instrumentenkunde*,

in German, by M. von Rohr and A. Kohler on "Microscopy with ultra-violet Light," and an abridged translation appeared in the 1905 Journal of our Society. This represented the most important advance within the period of my association with the Society and profoundly altered my outlook. There is no need to go into the possibilities opened up; they were, indeed, almost unlimited and have not yet been fully exploited. Certain hints of this advance in method had appeared but no practical realization came until Zeiss produced the complete apparatus that in its essentials remains unaltered to-day. The first objectives were quartz-fluorite combinations, but these were soon replaced by fused quartz monochromats, which are still unsurpassed. These objectives were noteworthy; their correction for spherical aberration was achieved by von Rohr by multiplying the air-quartz surfaces. There are still no other lenses in which this correction is so perfect. The complete equipment was expensive and so difficult to use that few mastered its manipulation. The records in our Journal show the steps that were taken to overcome these difficulties, but it was mainly at the National Institute for Medical Research in London that an efficient practical apparatus was evolved which remains in use to-day.

In February, 1906, Dr. Walter Rosenhain, F.R.S., exhibited a metallurgical microscope of new design which in its essentials embodied the modern "radial" principle. The stand was one of the most stable designed up to that time, being equally rigid whether in the vertical or horizontal position. It carried all the essentials for metallurgy and for mineralogy, and could also be used for ordinary purposes with transmitted light. In 1908 the death occurred of Dr. Henry Clifton Sorby, F.R.S., one of the Society's most distinguished Fellows. He was President in 1875–7 and delivered two addresses of a striking and suggestive character as well as making many other contributions to this and other societies.

In 1909 the death occurred of Henri van Heurck, of Antwerp, a distinguished Honorary Fellow, who was an authority on microscopy and successfully devoted attention to the resolution of diatoms. This subject has always been well to the front in the records of our Society; by some it is regarded as the keystone to the optical advances in the microscope during the past hundred years. There is no doubt the demands of diatomists did act as a stimulus to opticians, although it is difficult to regard any specific advance in optics as being due to their requirements.

Another distinguished Fellow, the Rev. W. H. Dallinger, F.R.S., died during the same year, having been President for the four years 1884–7 and Secretary from 1891 to 1907. He was well known as a member of the Wesleyan ministry, and as a popular lecturer on certain branches of biology he was unrivalled. The researches which made his name famous began about 1870, when, in conjunction with J. Drysdale, he published a series of papers on the "Life History of the Monads." Many of his experiments and observations bore directly on the question of spontaneous generation, and the results of these inquiries had a great share in determining the issue. This

was Dallinger's outstanding contribution to pure scientific inquiry. The work by which he is known to microscopists all over the world is his edition of Carpenter's *The Microscope and its Revelations*, published in 1891 and 1901, a book that will still be found in the hands of most microscopists at the present day. The concluding words of his presidential address in 1888 may well be quoted: "This and this only can lift a Society of this sort out of what I believe has ceased to be our danger, that of forgetting that in proportion as the optical principles of the microscope are understood, and the theory of microscopical vision is made plain, the value of the instrument over every region to which it can be applied, and in all the varied hands that use it, is increased without definable limit." An obituary notice and list of his published papers will be found in the Journal for the year 1909.

In 1910 E. M. Nelson, whom we have already had occasion to mention in the present address, published in the Journal a description and justification for his method of "Critical Microscopy," which in its essentials still remains the standard method of procedure, although variations in detail have been introduced. Thus it is no longer regarded as essential that an image of the radiant should be projected into the field of view, any part of a plane wave-front in which illumination is uniform may be treated as the illuminant. The essential, it is now thought, is that the objective shall be evenly filled with light for as much of its aperture as can be usefully employed. Nelson continued his contributions to the work of the Society, although his appearances at meetings became less frequent.

In 1912 the presidential address by H. G. Plimmer was noteworthy as it dealt with an essentially medical subject from a purely microscopical standpoint: rather an innovation at the time. Plimmer was a microscopist of the first order. I knew him well and admired his technical ability; a microscope as set up by him was always in perfect adjustment and the image he secured left no doubt in the mind of a competent observer as to its accuracy. In this year although the contributions to the Journal were not extensive, there were some papers of outstanding interest on diatoms and foraminifera by leading authorities. On the instrumental side fluorescence work claimed some attention; the apparatus designed at this time by some continental makers remains unaltered in principle at the present day.

A contribution of unusual interest was provided in the Journal by A. E. Conrady, who examined and published a considerable number of notes and manuscripts on optical subjects by J. J. Lister, which were left to the Royal Microscopical Society by his son, the celebrated Lord Lister. It is difficult to indicate in brief the subjects of these letters—they must be read in the original as published in the Journal. "Although it is impossible to secure to Lister the priority which he forfeited by refraining from timely publication, it is hoped," says Conrady, "that it will cause him to be included in the illustrious list of English amateurs who have made notable contributions to science." In September, 1912, the Lister Legacy was presented to the Royal Microscopical Society by Sir Rickman Godlee, President of the Royal College

of Surgeons, on behalf of the executors of Lord Lister, O.M. It comprised a bust, mounted on a pedestal, of Joseph Jackson Lister, F.R.S., the father of the testator and one of the founders of the Royal Microscopical Society, together with numerous manuscripts, unpublished papers, experimental lenses, test objects, and other things of interest made by Lister. The bequest was and still is regarded as one of the most important gifts to the Society. It is described in a paper read to the Society in February, 1913, by Edmund J. Spitta.

The Presidential address in this year was again by Plimmer, who selected a subject of supreme importance to all microscopists, the life and work of Anton van Leeuwenhoek. His introductory sentence is indicative of his purpose. "I propose to take as my subject the life-work of a man to whom microscopists especially owe an earnest reverence; but one of whom no picture nor written word is to be found in our Journal." Since that time an exhaustive and learned work on Leeuwenhoek has been published by Clifford Dobell which is a worthy tribute to the father of microscopy, but Plimmer's presidential address, with the title of *Bedellus immortalis*, forms a fitting introduction to the subject.

The period 1914 to 1918 was difficult, because of the World War, but the work of the Society continued without a break. The most obvious effect is shown by the reduction of the size of the volumes of the Proceedings, but there is no obvious change in the interest of the subjects discussed nor in the range covered. In the Journal of 1914 two important papers on the binocular microscope appeared, both of which might be read to-day, as no further advances have been recorded. The use of the binocular microscope is still limited, in part by the increased cost, but also because, apart from its undoubted comfort in use, it does not improve accuracy of image formation. Another important paper was by H. Lehmann on what he calls the "Luminescence Microscope," which surveys the whole subject of fluorescence microscopy. Under the Presidency of Mr. E. Heron-Allen, F.R.S., increased interest was aroused in the foraminifera, an arresting discussion ensuing between him and Prof. Ray Lankester which is reported in the Journal for the years 1916–17. In the latter year some increase in references to the bacteriology of war wounds may be noted.

The foundation of a Department of Technical Optics, in connection with the Imperial College of Science and Technology at South Kensington, is a matter of more than passing interest, although officially the Society was not involved. This development still expands, and has justified itself to the full in contact with the Northampton Polytechnic Institute in London, and other technical centres. The department is under the direction of a Technical Optics Committee, consisting of representatives of the Admiralty, the Army Council, the Ministry of Munitions, the Royal Society, the National Physical Laboratory, employers in the optical trade, glass manufacturers, and the Imperial College. The same Committee appointed by the L.C.C. also acts as an advisory Committee to that Council. One of our Fellows, Mr. Frederic

J. Cheshire, who at a later date was President of our Society, was appointed head of the new department, with the title of Director, and Professor of Technical Optics at the Imperial College.

The Journal for the years 1918–19 reached the limit of thinness, the number of papers presented falling to a low level, as might be expected with the conditions then prevailing; but in part the reduction in bulk was due to a change in the method of reporting the proceedings at the meetings, discussion being no longer reported fully, so the reduction of material was more apparent than real. As a result of war conditions there was a shortage of optical apparatus, and in consequence less appeared in the Journal in reference to such appliances.

A symposium was held in January, 1920, with the title of *A Symposium and General Discussion on The Microscope, its Design, Construction and Applications*. The Faraday Society, Royal Microscopical Society, Optical Society, and the British Science Guild, met in joint session at the rooms of the Royal Society, Burlington House, on January 14th of that year. The opening meeting was so large that numbers could not be accommodated. It was presided over by Sir Robert Hadfield, F.R.S., President of the Faraday Society, and the opening address was delivered by myself as President of the Royal Microscopical Society. This gathering and its associated meetings aroused widespread interest, but after this lapse of time it is possible to express doubt as to whether its purpose was fulfilled. A full report is contained in the Journal of the Royal Microscopical Society for 1920, and covers some 260 pages. One result, and that only transient, was an increased interest in the application of microscopical methods to various trades and industries.

The Journal for 1921 and 1922 has several papers of considerable interest on applied microscopy, and interest in some of these subjects continued into later years. Under the influence of Prof. F. J. Cheshire the year 1923 saw some increased interest in optical and instrumental subjects. His presidential address on the polarizing microscope provided an opportunity for an interesting résumé of a none too well understood branch of microscopy, illustrated by a number of striking demonstrations. It is interesting to recall that he suggested the direction in which experimental work should proceed, and, with remarkable foresight, urged that efforts should be made to provide artificial crystals to replace the expensive and increasingly rare natural products. Fox-Talbot, and, more particularly, Herapath, nearly succeeded in producing the required crystals, and Cheshire said, "I think their work should be continued." These words were prophetic, but, unfortunately, the problem was not solved in this country. As so often happens, a practical solution, based on our early pioneer experiments, has been found elsewhere. Cheshire's second address, published in the Journal for 1924, was on *The Design of a Petrological Microscope*, and is a sequel to his first address, with which it should be read.

There are few microscopes to which the term "new" may fully be

applied, but such a one is described by Mr. M. T. Denne in the volume for 1926. I must confess that this instrument always impressed me more than any other microscope I have ever seen, for it was designed by one who by training is an engineer, and to whom microscopy was little more than a hobby. The result is that Denne's microscope is not merely a modification of a previous type but is one so designed that the whole scheme provides a complete unit which needs no extraneous subsidiary appliances. The ordinary microscope limb is still there, but the illuminating and substage apparatus, whatever the type selected, is carried on an optical bench of rigid design. Perhaps the only criticism that can be offered is that with such a design a more than usually accurate, or at least a slower-moving, fine adjustment might easily have been embodied and would have been justified. The description should be read to appreciate the purpose of the innovations in design. Mr. Denne had already designed a photo-micrographic equipment which was outstanding in its completeness, but the microscope in particular remains as an example of a near approach to perfection. Perhaps its cost has prevented its more general adoption.

The year 1927 witnessed a change of format in the Journal, so that it now harmonized with similar scientific publications. It was a reform long overdue and the change has been fully justified. In this year there recurred a definite revival of interest in micro-metallurgy. This was due to the papers by H. Wrighton and Conrad Beck on the principles of illumination on which success in this somewhat difficult branch of microscopy is so largely dependent. These papers deal with the optical arrangements necessary for illuminating opaque surfaces; they do not treat the subject of polishing and etching, which is really a specialized branch of the subject dealt with in text-books on metallurgy.

The year 1928 witnessed a renewed interest in the subject of resolution in the microscope, and definite conclusions were arrived at which appeared to approach an agreed solution. This happened during my second period of occupation of the presidential chair. Of course there was discussion, but it was not so heated as some I can remember. Essentially it resolved itself into the question of the universal application of the *Abbe Diffraction Theory*. Would this theory account for image formation in the microscope under ordinary working conditions, whether using dark-ground or transmitted light illumination? The conclusion arrived at was summarized by Mr. H. Moore, D.Sc., in the concluding part of his paper, with which there was no serious disagreement. Mr. Conrad Beck, in his book on "The Microscope" (1924), drew attention to the fact that anything that can be resolved by transmitted illumination can be resolved by dark-ground illumination, and in general with much greater brilliancy because of the increased contrast between different parts of the structure. There is so much experimental evidence to support this contention that it is now accepted by most competent workers, although the Abbe theory does not cover such conditions. The Abbe theory undoubtedly gives a complete explanation of the appearances

seen when the object has a regular periodic structure and is illuminated by a cone of very small angle, but it is not applicable when the cone of illumination is large, and no proof of its applicability under these conditions has ever been given. So the deduction is arrived at that under the conditions of illumination commonly employed, and on which true image formation depends, the Abbe theory is no longer tenable. The paper should be read in its entirety ; it is most interesting and carries conviction.

Some further discussion took place in 1929, and there were several interesting papers, but it must be admitted that there was little to shake the general conclusions arrived at by Dr. Moore. Confirmation of this opinion was forthcoming from other competent workers, so that a complete theory of image formation in the microscope to cover all working conditions is still awaited with interest. Perhaps the newer theories of light transmission will open up possibilities at present unforeseen.

The year 1930 was a landmark in the history of the Society. Owing to changes at Hanover Square, where the Society had met for many years, it was not found possible to renew the lease, which was nearing its termination. After the discussion of many alternative schemes, an arrangement was made with the British Medical Association by which the Society was enabled to obtain suitable accommodation at their new premises, for offices, library, and instrument collections, and to use the Hastings Hall for meetings. This has proved entirely satisfactory, and although there was some feeling of regret at breaking with our old associations, it is admitted that the present scheme is an improvement from all points of view. The Society was fortunate that at such a critical time in its history the active direction of its affairs was in the hands of Dr. C. Tierney, who is still so engaged. Not only the arrangements for its new home but the difficult negotiations for relinquishing the lease on the Hanover Square rooms fell for the most part on his shoulders. Both were successfully concluded upon terms and conditions that the Society has had every reason to regard with satisfaction. At this period my rather disjointed review of the Society's activities ends, as I feel that the last ten years are sufficiently fresh in our minds to need no recounting and these reminiscences have been drawn out far beyond my original intention.

Although the subject must be treated briefly, it is of great interest to consider the changes that have taken place in our conception of the nature and transmission of light during the past hundred years or more. It was in 1690 that Huygens first put forward the wave theory of light which in its essentials forms the basis of all microscopical theory. Newton favoured it, but, as then formulated, it failed to account for rectilinear propagation and polarization. And so for another hundred years the undulatory hypothesis was held in abeyance and the corpuscular theory prevailed. The scheme to which there seemed to be the least objection was adopted for the time being ; opinion swayed according to circumstances.

The opening of the nineteenth century saw the end of the corpuscular

theory as it was then understood. The work of Young and Fresnel, which accounted for the phenomena of interference on which the formation of the microscopical image is so largely dependent, was so strongly in favour of the wave theory that all previous conceptions were superseded. The mathematical genius of Fresnel developed the undulatory theory to account for diffraction and rectilinear propagation, and later both experimentally and theoretically for polarization. This resulted in the generalization that propagation was by means of transverse vibrations. But the obvious question arose as to the nature of the medium in which these transverse undulations were transmitted. After Fresnel the theory of an all-pervading ether was postulated, and in this country Green and Stokes, supported by Kelvin, regarded this transmitting medium as an elastic solid, something like a tenuous jelly.

With such a doughty champion as Kelvin this theory held the field until the advent of Clerk Maxwell, with his electro-magnetic theory, which correlated light and electricity. This was probably the greatest conception of that century, but it still regarded the ether as the transmitting agent, although it offered no concrete conception of the nature of the ether itself. With the advent of Maxwell's electro-magnetic theory the individuality of light was largely lost and it became a part of the study of electricity, but in terms of image formation in the microscope we still have to regard transverse vibrations of some sort as essential. Kelvin sensed some difficulties in the existing theories, but the discovery by Röntgen of X-rays raised questions that could not be answered for some time, and indeed are not yet beyond discussion. We of to-day are probably no nearer a complete understanding of the nature of light or of its modes of transmission than were our predecessors; perhaps we discuss the question with even less confidence than they did. With the discovery that X-rays are similar to ordinary light, differing only in wave-length, the correlation of the phenomena observed became necessary. The main experimental difficulty was to obtain diffraction with X-rays, no ruled gratings giving the desired result; but with the discovery that natural crystals can behave as gratings these difficulties disappeared. X-rays were then proved to have all the characteristics of ordinary light. So far this simplification appeared to clear the air, but only for a time. Certain observed facts did not fit into any relatively simple wave theory, and so we come to the more recent discoveries on radiation in general.

The undulatory theory had always assumed light to be continuous in nature; waves were emitted continuously from any sustained light source. The quantum theory, which presumably overcame the theoretical difficulties encountered, was evolved by Planck. Energy, according to Planck, is not continuous but is emitted in finite bundles, or quanta. This was in effect a revival of the corpuscular theory, except that particles of matter were no longer the units—atoms or quanta of energy of definite magnitude replaced them. The nature of a quantum cannot here be defined, but appears to have associated with it the concept of periodicity enabling the phenomena of

diffraction and interference to be accounted as part of its make up. For the satisfactory understanding of light transmission the wave-front hypothesis is still tenable; for other phenomena the corpuscular quantum is preferable; so the two theories have to be unified to cover all questions at issue, and this is in process of achievement.

It is not possible to pursue this subject further, as it would involve too long a discussion of current physical development and take us much beyond the permissible limits of a brief account of the development of physical optics in relation to the optical theory of the microscope. No paper has been published in our Journal in which recent physical theories have been considered, the classical wave theory has not in principle been departed from, nor indeed does it yet appear to be necessary to modify our outlook. To those who wish to follow the current lines of thought on development in physics the annual *Reports on Progress in Physics* published by the Physical Society will provide an account of the most important theories and experiments, and a paper by Dr. H. Moore in the volume of our Journal for 1940 will clearly indicate the latest theories that have reference to microscopical optics.

I think we have now devoted enough attention to the past, so perhaps a few thoughts may be hazarded as to the future, speculative though they must be in these uncertain days. Analogy suggests that our future as a microscopical society will still in general be dependent on the development of physical optics, and on the widespread applications thereto in biology and cognate branches of science. The past hundred years have been fruitful enough. Can we, with any confidence, anticipate a continuance? To me the answer is emphatically, *Yes*. The past hundred years have been fully occupied in developing microscopy in terms of the visual images and the time has not been too long; we have left practically untouched the vastly greater field in which invisible radiations are the source of energy. We must remember that radiation is the keystone of all recent research, not only in microscopy but in general physics. In microscopy reduction of wave-length not only means proportionally increased resolution but the production of an image-picture, photographically or otherwise, that is in different terms from anything we have so far been familiar with. So that on each side of the visible spectrum we have a range of wave-lengths ready to be utilized but with different characteristics. On the infra-red side the possibilities are limited, but the shorter wave-length range, beginning with the ultra-violet, opens up a vista that has hardly any limit. More than half of the years I have been connected with this Society I have devoted to microscopy with ultra-violet light. The results are small compared with the possibilities, but I have been almost alone; few have taken more than a passing interest in an admittedly difficult line of work. Perhaps the cost has hampered some, but I am doubtful whether that is the real cause of the apparent lack of interest—money has been found for less promising purposes. Perhaps a biological problem of sufficient importance has not been waiting a solution.

Biology is the science which so far has made the most important use of the microscope, but with the new world and the new problems opened up by the recognition of viruses this may all be changed. What is the limit of smallness that can be assigned to a living unit? Are we justified in regarding virus bodies as living, or are we to look on them as the bridge connecting the living and the non-living?

These are indeed speculations, but the next few years will almost certainly find an answer that will be different from our expectations. That the microscope, whatever form it may assume, will contribute something to the answer I am confident, and it may, in sufficiently competent hands, provide a solution. But this is a limited outlook, although it is important enough in itself. If experience is any guide, the multifarious byways will provide a constant succession of problems, each as interesting and absorbing as anything the past has offered. Even the interpretations of the images provided by the range of radiations it will be possible to utilize will in themselves provide the microscopist with the work of an ordinary lifetime. But there is another direction in which the possibilities cannot at present be estimated. The electron microscope is already in being, providing images of suitable objects as much as one thousand times smaller than anything we have hitherto observed. This subject may at first glance appear to be beyond the interest of microscopists, but in its essentials the instrument has arisen from and is based on the principles of pure geometric optics. The pages of our Journal already provide papers by recognized authorities to indicate the work on design and construction in progress. Actually the advance has been greater than indicated, but the disclosure of details is hardly possible at present. In whatever direction our thoughts may wander it is impossible to overestimate the potentialities of our subject.

The passing of our centenary has not been ostentatious, the occasion has lost some of its significance, but the results obtained in the past will not be buried, their foundations are too well and truly laid. The future of microscopy will doubtless provide as solid a record of achievement as these past hundred years have seen, and the workers in the days to come will look with pride on the structure that has already been built, almost entirely on the efforts of these early seekers after truth who regarded no effort too great so long as their purpose was fulfilled.

In conclusion I am quoting some remarks made by the President of the Royal Society, Sir William Bragg, O.M., as an introduction to a recent article in *The Times*. He expresses so clearly and eloquently the thoughts that have passed through my mind that I cannot hope to equal, much less to improve upon, them.

> "Scientists have so many new tales to tell that they are often supposed to pay little regard to that which is old. It is a fundamental mistake. Science is built on the accumulation of experiences, and every scientist knows that he must not base his conclusions only on the last few experiments in the laboratory. He must take into account all that has been

done before. History is to him a very real thing; tradition is priceless, because there is no substitute for it. Practice is based on the experiments of innumerable years, and even when there are no written records to be consulted, the behaviour of men, the thoughts they have turned over and sifted, the knowledge they have gained in ages of trial and error are all of value; they are not to be thrown aside in favour of the last galvanometer reading. When a scientist records a discovery he only makes a new entry in an old book."

The aptness of these remarks will need no stressing. They apply in their spirit and intention to the record and tradition of the Royal Microscopical Society.

APPENDIX.

THE HISTORY OF THE ROYAL MICROSCOPICAL SOCIETY.*

By A. D. Michael, F.L.S., etc.

(*Read January 16th*, 1895.)

A presidential address is usually a review of something. This Society has now existed for over half a century; I think, therefore, it is a time when we can afford to devote part of a single evening to a subject which, although not strictly to be called scientific, may probably interest you; namely, a review of the history of the Society itself.

If any one of you will leave this West End abode of science, and crossing the business parts of the City will make his way eastward as far as Tower Hill, and leaving Tower Hill at Sparrow Corner will pass along Royal Mint Street, he will find himself in Cable Street, St. George's in the East. I cannot promise that he will be struck either by the quietness or the cleanliness of the locality; the shadow of the neighbouring docks is on it, and its principal features seem to be rag and bottle shops, marine store dealers, and street stalls; but if he turn to the right down Shorter Street he will suddenly emerge in a much quieter spot; in front of him will be what has once been a considerable space of green, but is now chiefly occupied by St. Paul's church for seamen. The "Infant Nursery" belonging to that excellent institution will be exactly facing him, but the foundation stone of that building was not laid until 1872, so at the time I am going to speak of the Green still existed; and although a church stood upon it, it was the Danish church, in which was a royal closet specially reserved for the King of Denmark when visiting this country, opening by two sash windows, one looking on to the pulpit and the other over the congregation. Beyond the fine old trees which surrounded the church was a somewhat famous spring well. The place, I fear, will not now impress our traveller as having either a scientific or a fashionable aspect; but if he passes the Catholic Seamen's Club and observes the houses surrounding the enclosure he will notice that many of them are old, and have been good, substantial brick houses, pleasant enough to live in; and that some bear considerable remains of artistic ornament. Following round the enclosure he will find a house which certainly looks as if it had stood there for considerably more than half a century; at the present moment its bell-handles are broken relics, and the ridge-tiles of its roof look as if they would

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be better for a little repair ; but this house is No. 50, Wellclose Square, and there, in the year 1839, lived Edwin J. Quekett, F.L.S., and in its drawing-room, on September 3rd of that year, seventeen gentlemen assembled "to take into consideration the propriety of forming a society for the promotion of microscopical investigation, and for the introduction and improvement of the Microscope as a scientific instrument."

Probably few of my hearers have ever heard of Edwin Quekett, he has been so entirely overshadowed by the fame of his younger brother John ; but Edwin Quekett was a man of considerable scientific reputation, and had he lived longer he would probably have increased it. Born at Langport, in Somerset, in September, 1808, he commenced his studies for the medical profession at University College when he was just twenty ; there he gained one gold medal in anatomy and physiology, another in practical anatomy, and a silver medal in chemistry, besides a certificate of honour in every class he attended. He practised his profession at Wellclose Square, but he was chiefly known as lecturer on botany at the London Hospital medical school. He contributed numerous scientific papers to the various societies and journals of his time, including six important papers to our own Transactions. He died at his house in Wellclose Square on June 20th, 1847, under the age of thirty-nine.

Seventeen men did not meet at Edwin Quekett's house on that September 3rd, 1839, by pure accident ; coming events had thrown their shadows before ; nor was Edwin Quekett the only man of science, or the only one of our original members, who lived in Wellclose Square. At No. 57 lived the Rev. William Quekett, who was the original of Charles Dickens' sketch in the second volume of *Household Words*, entitled "What a London curate can do if he tries." At No. 46 lived Charles Foulger ; at No. 45 lived Edward Newman, the well-known entomologist, and for so many years editor of the *Zoologist*, whose connection with microscopy included not only his original membership of this Society, but also his famous diatribe upon the ignorance of microscopists apropos of someone having mistaken the curious eggs of the Stone-Mite for a new fungus, and called it *Craterium pyriforme* ; and last, but by no means least, at No. 7 lived Nathaniel Bagshaw Ward, the botanist and inventor of the Wardian case. He was born in 1791, at Plaistow, in Essex, where his father practised as a medical man ; the son was brought up to the same profession and practised it at Wellclose Square, where he made his discovery of the glazed cases for the transmission and growth of plants, which were the means of introducing the tea-plant into Assam, the cinchona into India, and which are used to this day whenever it is desired to import new plants into this or other countries—not to speak of their having rendered life somewhat more pleasurable to thousands of dwellers in cities who have a taste for ferns and mosses. Ward erected a large Wardian case on his staircase at Wellclose Square, and a Tintern Abbey window was modelled for it by Edwin Quekett. When he left the square and went to live at Clapham Rise, Ward took this window away with him, and it stands to this day where Ward put

it, namely, in the rockery round the water-lily pond at what is now No. 303, Clapham Road, where lives our respected Past President, Dr. Braithwaite, and it may not be unknown to you that Mrs. Braithwaite was once Miss Ward.

Ward was one of the most active founders of this Society; he was its first Treasurer and occupied that position for twenty-three years; he retired in 1862, and died on June 4th, 1868, at the age of seventy-seven. Ward and Dr. Bowerbank practically kept open house to microscopists and other men of science, and a number of microscopists, whom Bowerbank called his "Band of Brothers," used to meet frequently at these two houses. It was after one of these gatherings, assembled to greet Ehrenberg, that Bowerbank is said to have exclaimed to the Rev. J. B. Reade, "God bless the Microscope, let us have a Society." The matter was broached at the next meeting in Ward's drawing-room, and it was in consequence of what took place there that those seventeen gentlemen came to Edwin Quekett's house on that September evening some five-and-fifty years ago.

The time was propitious; ideas of the improvement of the microscope and microscopical science had been in the air for some little time, and the Society was becoming a want. In January, 1830, Mr. Lister had published his epoch-making paper "On the Improvement of Achromatic Compound Microscopes, announcing the discovery of two aplanatic foci in a double achromatic object-glass, upon which, together with the practical directions of the author, Messrs. Powell, Ross, and Smith (names not unknown to us to-day), worked so successfully. In 1832, Mr. J. T. Cooper's suggestion of using Canada balsam for mounting objects had been put in practice by Mr. Bond, and a first notice of it had appeared in print in 1835, in a book by Mr. Pritchard entitled "A list of Two Thousand Microscopic Objects"; and in 1837 Andrew Ross had suggested the correction collar.

Among the seventeen assembled at Edwin Quekett's house we find, in addition to that gentleman himself and Ward, the names of Mr. Bowerbank (not Dr. then), Dr. Farre, George Jackson, and the Rev. J. B. Reade, all of whom became Presidents of the Society, and also Lister himself, George and Conrad Loddiges, the nurserymen, and Cornelius Varley. Joseph Jackson Lister, F.R.S., was born in London on January 11th, 1786; his parents were members of the Society of Friends, to which he also belonged throughout his life; at the age of fourteen he left school to assist his father in the wine trade. His tastes were shown early, for as a little child he found out that if he looked at distant objects through air-bubbles in the window-pane the vision of his eye (then myopic) was improved—a subject which he subsequently worked out; it was not, however, until 1824, when he was thirty-eight years of age, that he turned his attention to object-glasses. The history of his optical researches in this and other subjects, culminating in the great paper of 1830, which will be found in our Transactions for 1870, p. 134, is from the pen of his still more famous son. It has been said of Lister, by one well fitted to judge, that "he was the pillar and source of all the Microscopy of the age." He died at Upton House, Essex, in October, 1869, at the age of eighty-four.

The Loddiges were a great deal more than ordinary nurserymen: George was one of the most liberal patrons and most skilful users of early achromatic objectives; he was his own architect for his great palm-house at Hackney, which was built by his own workmen. In his publication, "The Botanical Cabinet," eight hundred and eighty-nine of the figures were drawn by himself; his collection of casts of antique gems was about two thousand, principally taken by himself; and his collection of humming-birds, on which he intended to publish a book, was at the time unrivalled.

Cornelius Varley will also be a name well known to you; he was a very active early member of this Society, and his communications, illustrated by rough but effective drawings, will be found frequently in our Transactions.

It will be remembered that the seventeen had met "to consider the propriety of forming a Society for the promotion of microscopical investigation, and for the introduction and improvement of the Microscope as a scientific instrument." The result was a resolution that such a society should be formed, and that a Provisional Committee (Bowerbank, Lister, G. Loddiges, E. Quekett, Reade, Solly, and Ward) be appointed to carry this resolution into effect.

This Committee held meetings, drew up a set of rules, adopted the name of "The Microscopical Society of London," which was devised by Bowerbank and Reade, and arranged to hold a public meeting at the rooms of the Horticultural Society, which were then at No. 21, Regent Street, on December 20th, 1839. At this meeting Prof. Richard Owen took the chair and was elected President; Ward, Treasurer; and Dr. Arthur Farre, Secretary. Forty-five gentlemen inscribed their names in a book as original members, and it was resolved that all who joined before January 29, 1840, were to be considered original members.

No more fitting first President than Prof. Owen could possibly have been found: his is a name of world-wide reputation; but at the time of his death, which occurred since I have occupied this chair, so many able memoirs of him appeared in print, and so full a biography has just been published that I hardly think it would be desirable for me to enter here upon any lengthy summary of his history or achievements; but I may remind you that when he became our President he was not the venerable and striking figure which we used to see near the Sheen Gate of Richmond Park, and which made so deep an impression on all present when he received the first Linnean gold medal. Born at Lancaster in July, 1804, he matriculated at Edinburgh in 1824, and in 1825, when he was just twenty-one, he paid that visit to Paris which enabled him to make Cuvier's acquaintance, and probably greatly influenced his future life. He became one of Abernethy's dissectors at St. Bartholomew's Hospital, and it was upon Abernethy's suggestion that Owen was first employed in 1828 to catalogue the Hunterian collection at the College of Surgeons, with which institution he was connected until 1855. When he took the chair at our first public meeting Richard Owen was a man of thirty-five, in the full tide of work and vigour; he was already a Fellow

of the Royal Society, Lecturer on Comparative Anatomy at St. Bartholomew's, and Hunterian Professor at the Royal College of Surgeons; he had just completed his catalogue of the physiological specimens in the Hunterian collection, in five quarto volumes, and was commencing his great work on the study of teeth, but the larger number of the three hundred and sixty papers which he contributed to the Transactions of various learned Societies, and which will be found duly enumerated in the Royal Society's catalogue, and at the end of vol. ii of Owen's biography, were yet to come. He remained our President for two years, was a regular attendant in the chair, and took a deep interest in the welfare of the Society.

On January 29th, 1840, Owen was in the chair at the council meeting when Bowerbank reported that, in addition to the forty-five members who had joined at the first meeting, sixty-five more had joined the Society; he also reported that the Society had made its first purchase; it is rather amusing to find that this was a diamond and cutting-board, to cut glass slips for the use of the members, the Provisional Committee having fixed on the sizes of 3 in. by 1 in. and 3 in. by 1½ in. as those to be adopted.

At this moment Schleiden was commenting upon the paucity of British microscopical research, and attributing it to the want of efficient instruments, not knowing that an Association was then forming which was to raise British Microscopes to probably the first position in the world.

On February 29th, 1840, it was decided to accept the offer of the Horticultural Society to allow the Microscopical Society to hold its evening meetings at their rooms, 21, Regent Street, for a payment of £20 a year.

It would seem that thus early in the Society's career a question arose which has troubled many others, for we find a resolution of March 18th, 1840, "That it did not appear desirable at present to act upon the rule relating to the holding of soirées at which ladies should be admitted."

On January 27th, 1841, Mr. Daniel Cooper submitted a prospectus of a proposed Microscopical Journal, and asked permission to publish abstracts of the papers read at the meetings of the Society, offering to make the Journal serviceable to the views of the Society; this request was granted, and thus the Transactions of the Society first appeared in print, and thus commenced the connection of the Society with Cooper's, afterwards Cooper and Busk's, *Microscopic Journal*, published by one who has done good service to English science, and whose face is still sometimes seen with pleasure at the Linnean Club, viz. the veteran John Van Voorst.

On February 15th, 1841, the Society held its first annual meeting, and it was then reported that it had one hundred and seventy-seven members; it is a curious evidence of the change of times to find that fifty of these resided in the City of London. The recognition of the scientific importance of the Society is shown by the fact that twenty-two of the members were Fellows of the Royal Society, and its wide range of interest by the fact that the list included such widely different names as Richard Beck, Thos. Bell, Professor of Zoology at King's College, John Birkett, of Guy's, George Busk,

Sir James Clarke, John Edward Gray, Keeper of the Zoological Department of the British Museum, Chas. Hullah, the musician, John Kippist, the librarian of the Linnean, Dr. Lindley, the Marquis of Northampton, then President of the Royal Society, Andrew Ross, Dr. Sharpey, John Tomes (not Sir John then, and miscalled Thomas in the lists of members), Van Voorst, and Erasmus Wilson. In spite of all this the new-comer was perhaps not universally received with special cordiality, for we find Owen in his first address combating the idea that the Microscopical Society would deprive the older Societies of biological papers.

On February 17th, 1841, the propriety of appointing a librarian and curator was discussed, and it was suggested that John Quekett might accept it as an honorary office. On March 17th the first number of the *Microscopic Journal* was presented by the editor; on June 23rd, Dr. Farre resigned the secretaryship, and John Quekett was appointed to that office, which he held for nineteen years. It is odd that these are the first mentions of John Quekett in connection with this Society; he is not among the original seventeen, and yet he must have been residing in the house at the time, and as he is marked as an original member he must have joined the Society before January 29th, 1840. This is, I think, the appropriate place to say a few words about him, for although he was eventually elected President, yet it was a mere compliment at a time when his health would not allow him to perform the duties of the office. It was as Secretary that he laboured so earnestly to promote the welfare of this Society, and it was during his secretaryship that he contributed the twenty-one papers, including some of his most important, which adorn our Transactions.

Probably no sounder or more earnest student of nature ever lived than John Thomas Quekett, who was born on August 11th, 1815; he was the youngest son of the head-master of Langport Grammar School; his earliest tastes were for entomology, and accompanied by his brothers Edwin the botanist and Edward the ornithologist, and by his sister, who in later years drew so many of his diagrams, he used to wander about the Langport woods in search of those treasures with which the young people filled their father's house. So early did he turn his attention to the Microscope, that at the age of sixteen he delivered a course of lectures at Langport on microscopical science, illustrated by diagrams and by a microscope manufactured by himself out of a roasting-jack, a parasol, and a piece of brass which he bought at a marine-store dealer's and hammered out. He was described as "strangely sedate, careless of his appearance, heedless of conventionalities, and unattracted by the ordinary amusements of children." Being intended for medicine he was sent to London, apprenticed to his brother Edwin, and entered at the London Hospital; he became a M.R.C.S. in 1840, and in the same year competed for and obtained the studentship in human and comparative anatomy, then recently established. When the studentship expired in 1843 he became Assistant Conservator of the Hunterian Museum at the College of Surgeons; in 1844 he was appointed by the Council "to deliver

annually a course of demonstrations with a view to the exhibition and connected description of the collection, and the explanation of the method and resources of microscopical study." On the retirement of Owen in 1856 Quekett was appointed his successor and also Professor of Histology; these offices he held up to his death. He was elected a F.R.S. in 1860, shortly before his death; he made over sixteen thousand preparations for the histological collection of the College of Surgeons, which he practically created. In 1848 he published his celebrated "Practical Treatise on the Use of the Microscope," a German translation of which was published in 1850; his other important works are too numerous for me to mention here; yet he died at the early age of forty-six, at Pangbourne, on August 20th, 1861, and for some time previously had been in very bad health; and few men spent so much of their time in helping others.

It had been anticipated that probably the construction of the Microscope would be the first subject which would attract attention, but this turned out not to be so; such was the activity of biologists that, although the number of papers presented to the older Societies was not diminished, yet those that flowed in to the new Society from men of first-class standing were at first almost wholly biological; the equally, or perhaps even more, important optical and mechanical papers came more slowly and later.

In this year (1841) we first find the name of Michael Faraday among our Members, and in September and October of this year it was determined that the Society should publish its Transactions in royal octavo, as it does to-day; this arrangement lasted for ten years. Those who will look at Leonard's illustrations to Quekett's papers on the intimate structure of bone, on the capillaries in the gills of fishes, and on the vascularity of the capsule of the crystalline lens in Reptilla, or at George Busk's illustrations to his papers on *Notamia bursaria* and *Anguinaria spatulata*, or Warren de la Rue's to his paper on the markings on the scales of *Amathusia Horsfieldii*, may not improbably think that the advance in illustration up to this time is more conspicuous in quantity than in quality; but this I fear is the same with almost all English scientific drawings; those of forty or fifty years ago compare not unfavourably with those which are now appearing.

On February 15th, 1842, Owen retired, and Dr. John Lindley was elected President; he was the son of a nurseryman, and was born at Catton, near Norwich, on February 5th, 1799, and educated at the Grammar School of Norwich; which he left at the age of sixteen, and was then employed for three or four years at his father's nursery. His father failing in business, Lindley was thrown on his own resources. He proceeded to London in 1819, and obtained from Sir Joseph Banks the position of his assistant Librarian. At this early age he began the long series of works with which his name is identified by the publication of a translation of Richard's *Analyse du Fruit*, made at one sitting, which, however, lasted two days and three nights.

In 1822 Lindley became Garden-Assistant-Secretary to the Horticultural Society, and in 1826 he became sole Assistant Secretary. He was elected a

F.R.S. in 1828; became in 1829 Professor of Botany in University College, which appointment he held for upwards of thirty years. His "Synopsis of the British Flora," published in 1829, was followed by an "Introduction to the Natural System of Botany," in 1830, which ultimately took the form of "The Vegetable Kingdom," probably the best known of his works. In 1831 he produced, with the aid of Mr. Hutton, the well-known "Fossil Flora of Great Britain," and he published numerous other general works and papers. In 1857 the Royal Medal of the Royal Society was awarded him. He was a juror of the Great Exhibition of 1851, and undertook the Colonial Depar-ment of that of 1862; but the strain was too much and he never recoverep from it. He died on November 1st, 1865.

During Lindley's presidency we find a committee, consisting of Owen, Quekett, and Rymer Jones, appointed to investigate the structure of shell; we also find for the first time the name of Edward Forbes, who soon became a member of the Council and a contributor to the Transactions; and then was held the Society's first *soirée,* where twenty-one Microscopes were exhibited, which was then thought a great thing.

On February 15th, 1844, Lindley retired, and Thomas Bell was elected his successor; he was born at Poole in Dorsetshire on October 11th, 1792. His father was a surgeon, and he was educated as a surgeon-dentist. In 1815 he commenced lecturing at Guy's Hospital on the anatomy and diseases of teeth; he was appointed dental surgeon, and also lectured on comparative anatomy at the same hospital; and in 1836 he was appointed Professor of Zoology at King's College. He was President of the Linnean Society from 1853 to 1861. In 1828 he became a F.R.S., and was one of the Secretaries of the Royal Society from 1848 to 1853. Bell's treatise on the anatomy, physiology, and diseases of the teeth appeared first; his monograph of the Testudinata in the years 1836–42, his well-known works on British Quadrupeds and Reptiles in 1837 and 1839. He was joint editor with Forbes of Burmeister's Trilobites for the Ray Society, and joint author with Owen of the fossil Reptilia of the London Clay for the Palæontographical Society, which also published his "Fossil Malacostracous Crustacea"; his "British Stalk-eyed Crustacea" appeared in 1853. He described the "Reptilia of the Beagle" for the Government, and also the collections formed by the "Assistance" during the search for Sir John Franklin. In 1866 he retired from his profession, and purchased The Wakes, Selborne, from the grand-nieces of Gilbert White. The rest of his life was devoted to editing the 1877 edition of White's "Selborne." Bell died on March 13th, 1880, at the age of eighty-eight.

We find Bell in his annual addresses impressing two things upon his hearers: firstly, the extreme importance of using the Microscope more in pathology; secondly, the great desirability of cheap Microscopes—a favourite subject with the Society and its successive Presidents, who worked earnestly at it for over a long period with great ultimate success; although possibly some people might be inclined to think that Microscopes were cheap enough

already, for Ehrenberg, who was the first Honorary Fellow of this Society, tells us that the instrument which he carried half over the world, and with which he made most of his wonderful discoveries, was purchased in the streets of Berlin for thirty shillings.

On February 11th, 1846, Bell retired, and Dr. James Scott Bowerbank was elected in his place.

It has been said of Bowerbank that in science he was an amateur very near to the border of the professional; he was one of the founders not only of the Microscopical Society, but also of the London Clay Club, which gave birth to the Palæontographical Society, of which he was first Secretary and then President, and also of the Zoological Society. He was elected a F.R.S. in 1842, contributed many valuable papers to our Transactions and to those of the Royal, Linnean, Zoological, and Geological Societies; but he is probably best known by his great work on the British Spongiadæ published by the Ray Society. Bowerbank was born in Sun Street, Bishopsgate, in July, 1797, and succeeded to his father's distillery there, in which he was an active partner until 1847. He lectured publicly upon botany and upon human anatomy. Bowerbank's weekly receptions of microscopists and men of science have been before referred to; he continued them at his new house at No. 3, Highbury Grove, after he had left Islington (where he lived when the Microscopical Society was founded). No man ever was more anxious to communicate his knowledge and exhibit his scientific treasures to those who had tastes similar to his own; but he was not equally desirous of acting as showman to those who did not take an intelligent interest in them. I have been told, from what should be a trustworthy source, that Bowerbank kept a small collection of showy and striking slides in a special box which he called his "goodness gracious box," and that if anyone whose tastes he did not know wanted to see through his microscopes, he would show them some slide having plenty of information in it; if the visitor asked some intelligent question about structure the host was delighted to go on showing and explaining; but if the first remarks were unappreciative Bowerbank would hand him over to someone else to exhibit "the beautiful things in that box." Bowerbank has passed away from us, but the two classes into which he divided slide-seeing humanity still exist, the lovers of structure and the lovers of the "goodness gracious box" are with us to-day; personally I do not see why the latter should not get all the pleasure they can from the face of Nature. When Carpenter wrote somewhat contemptuously of "mere beauty," he, to my mind, as much missed one of the most charming aspects of Nature, as did a certain well-known artist, who when I was about to explain to him the structure of the object he was looking at, said, "I do not want to know how it is made, I am satisfied with the beautiful exterior. If I see a beautiful scene at a theatre, I do not want to go on the stage and examine all the cardboard and goldleaf that it is composed of." It seems to me that he who can admire both the structure and the beauty to the eye is the truest student of Nature and derives most pleasure from the study.

If any jealousy of the new Society ever existed it must have died out before 1848, for in that year we find the Linnean Society inviting the Microscopical to join them in their annual dinner.

In this year George Busk became President; he was an original member and a frequent contributor to the Journal. He was born in 1807, and was the second son of Robert Busk of St. Petersburg. He became a M.R.C.S. in 1830, and was appointed surgeon to the seamen's hospital ship *Dreadnought*; in 1856 he resigned this appointment to devote himself to scientific work. He became President of the Royal College of Surgeons in 1871. Besides papers contributed to various scientific societies and journals, he wrote the descriptive catalogue of the Marine Polyzoa in the collection of the British Museum; the article "Polyzoa" in the *English Cyclopædia,* which contained the first satisfactory attempt at a classification of the group; and the "Monograph of the Fossil Polyzoa of the Crag" for the Palæontographical Society; and he translated Steenstrup's "Alternation of Generations" for the Ray Society. In 1864, he and Dr. Falconer went to Gibraltar to investigate the ancient cave-fauna there; and finally he examined and described the Polyzoa collected during the voyages of the *Rattlesnake* and the *Challenger*.

It was during Busk's presidency in 1848 that we find in our Transactions a paper by Mr. Warrington, "On a new method for the mounting of organic substances as permanent objects for microscopic investigation." The medium recommended is "the liquid known under the name of glycerine"; and just at the end of his presidency that we find there Gosse's well-known paper "On the architectural instincts of *Melicerta ringens*."

Dr. Arthur Farre, F.R.S., who became President in 1850, was an original Fellow and first Secretary of the Society. He contributed several papers to our Transactions, but his fame was rather medical than microscopical. He was the fifth son of Dr. John Richard Farre, and was born in 1811 in Charterhouse Square, and educated at Charterhouse. He was Abernethy's prosector, lecturer on comparative anatomy at St. Bartholomew's in succession to Owen, and Robert Ferguson's successor in the chair of obstetric medicine at King's College. It was during his presidency that we find the first paper in our Transactions by Mr. Wenham, whose long series of inventions and improvements in the Microscope and its apparatus, which will be chiefly found in the pages of our Journals, have been of such material benefit to almost all workers with our favourite instrument. This list of inventions is far too long for me to attempt to refer to them to-night, but I may remind you that what is now known as the Wenham prism for binocular Microscopes is figured in our Transactions for 1861. During this presidency we also find the first paper by Mr. Sorby, who subsequently became your President, and by Prof. Huxley, who became a member of the Council in 1857.

In 1852 George Jackson, well known to microscopists by the Jackson-Lister form of Microscope, became President. Shortly after Mr. Jackson's election an offer was received from Dr. Lankester and George Busk that they would start and edit a journal, to be called the *Quarterly Journal of Micro-*

scopical Science, in which they would publish the Society's Transactions, and supply copies for the members, if the Society would contribute a certain sum towards the expenses; this offer was accepted, and the arrangement continued down to the end of 1868, and although the growth of both the Journal and the Society produced financial difficulties, which finally forced a severance of the connection, yet the journal exists to-day in the enlarged form in which it is so ably conducted by Dr. Lankester's son. The new journal did not improve the Society's finances, and consequently it was rather with dismay that the Council, about this time, received a notice from the Horticultural Society that they must raise their rent; the Society consequently removed to the rooms of the Chemical Society at 5, Cavendish Square; but these rooms were found so inconvenient that after a year the Microscopical moved back to the Horticultural Society's rooms, where they remained until the end of 1856, when the Horticultural Society sold its house in Regent Street. Then commenced the connection of the Microscopical Society with King's College, which lasted until when, King's College not being able to afford them accommodation any longer, the Society removed to its present rooms in Hanover Square.

At this time (1853) we find the first contribution to our Transactions by Wheatstone, which I may stop to notice, for probably there never existed a mind more teeming with scientific constructive invention than that of the inventor of the electric telegraph.

It may be thought that eminent scientific men are not invariably the best men of business when we find that up to October 26th, 1853, no minute book of the meetings of the Society had been kept; at that date one was started, and an endeavour was made to write it up for the back period, which was only partly successful, for we find in it an entry, " The minutes of meetings between 17th January, 1844, and 26th October, 1853, are lost."

Dr. Carpenter succeeded Jackson as President. He is, of course, a leading name in microscopy, but his work continued up to such a late period, and he and it are so well remembered by almost all of you, that it would be idle for me to speak of it at any great length to-night. George Shadbolt was the next, and I believe that he is still one of our members. It was during his presidency that the Council proposed and successfully carried out the idea of having the screw known as the " Society's screw " for the attachment of objectives, so that they might be interchangeable between Microscopes, which has been so great a boon to all English microscopists. It is perhaps to be regretted that the Society's later efforts to make other pieces of apparatus interchangeable did not meet with equal success. About the same time Maltwood read his paper, " On a new form of Object-finder," which has been of considerable service, even if somewhat superseded now.

In 1858, when Dr. Lankester became President and Jabez Hogg was first elected on the Council, the Society possessed a library of sixty-eight works; somewhat a contrast to its present condition. It was towards the end of this presidency that there appeared in our Transactions the first of that long

and admirable series of papers on the Diatomaceæ, by Dr. Robert Greville of Edinburgh, which do so much credit to the author and to our Transactions. He was a man of many activities, and I can well remember him in his latter years, polished and courtly in manner, a leader in Edinburgh society, and in the charitable and religious societies of that city he was enabled to obtain material for his search after diatoms from the missionaries in most wild parts of the world. He wrote on ferns in conjunction with Hooker, and on seaweeds alone; he was a good and eager entomologist in almost all orders, an excellent landscape painter in oils, and probably the beauty of his scientific illustrations has not ever been exceeded. Lastly, he was a remarkable pedestrian, and on one occasion walked 300 miles in a week.

In February, 1860, John Quekett's failing health compelled him to resign the secretaryship, and G. E. Blenkins, who remained a member of the Society until his death a few months ago, and M. J. Legg were appointed joint secretaries. Quekett was elected President without his knowledge, a letter from him asking to be excused on account of his health having arrived too late. But his election was a mere compliment; he was too ill to occupy the chair, and he never did so. At his request he was not re-elected, but during his year the number of members of the Society for the first time exceeded 300.

Quekett died in August, 1861, and there was a sale of his scientific effects, amongst which was a large and interesting Microscope by the celebrated Benjamin Martin, supposed to be of the date of 1770, and to have been made for George III, which is described in a paper by Williams in our Transactions in 1862. The Secretary of the Society was authorized to bid for this instrument up to £21, and the Library Committee to buy books at the same sale to the extent of £20, but it was thought that the Society could not afford this expenditure, and it was resolved to raise it by a private subscription, which should be extended so as to raise a fund, the interest of which should be devoted to providing a medal, to be called the "Quekett Medal," and given from time to time to those members of the Society who had best promoted microscopical science; afterwards it was decided not to confine it to members. The fund was raised, and in 1863 a committee was appointed to consider what steps should be taken with regard to striking the medal, and it was decided that the medal should be annual. In January, 1864, Dr. Carpenter suggested that the medal should be given for the best cheap microscope. Finally, advertisements were issued inviting competition in four classes of cheap Microscopes, the lowest to be a simple Microscope, and the lowest compound three guineas and the highest ten guineas. None of the three principal makers competed, and nothing sent in for the simple class was considered worthy of the medal. Eighteen instruments were sent in in the other classes to the rooms of the Astronomical Society, where the judging took place; those of Mr. Wheeler were found so superior to all others that all three medals were awarded to him. They were, however, so far superior that the Council called Mr. Wheeler's attention to the fact that the conditions were that equally good instruments should be supplied to the public at the

prices named; thereupon Mr. Wheeler refused to accept medals in any but the lowest class, whereupon the Council revoked the entire award, and the three silver medals which had been struck remain in the Society's cabinet to this day.

Nothing more was done in the matter until 1874, when a committee was formed to consider "what use could be made of the Quekett medal," and they decided to institute an annual Quekett Lecture, for which the medal should be given; bronze medals were struck, and Sir John Lubbock was invited to deliver the first lecture. He did so, and the highly interesting lecture, which we most of us remember, will be found in our Transactions for April, 1877. Difficulties arose in attempting to continue these lectures, and they were allowed to lapse, so that the only Quekett medal ever issued was that presented to Sir John Lubbock. In 1879 it was decided to expend part of the fund in the purchase of books, and to invest the balance, and apply the interest from time to time in the same manner.

It was not until 1865 that the question of obtaining a Royal Charter of Incorporation for the Society arose, and in 1866 Her Majesty was graciously pleased to grant the existing Charter and to consent to the word "Royal" being added to the name of the Society; the Prince of Wales became Patron and the Society assumed its present form.

In 1867 Blenkins resigned the secretaryship, and Jabez Hogg was appointed in his place, who retained the office until 1873, when he was succeeded by our friend Prof. Chas. Stewart.

At the end of 1868 the connection with the *Quarterly Journal of Microscopical Science* ceased, and a new journal called the *Monthly Microscopical Journal* was started, edited by Dr. Henry Lawson, and published by Messrs. Hardwick and Bogue; this continued until 1878 when, the number of Fellows having risen to over four hundred and fifty, the Society reverted to its original plan of publishing its own Transactions; and the new Journal was started, which was soon edited by Mr. Frank Crisp, in whose hands it became so great a credit both to the Society and the editor, and a publication of such great utility to microscopists and biologists generally.

We have now arrived at modern times which we almost all of us remember well, and at what is practically the present position of the Society, therefore I do not propose to carry this history any further; but I will consider for a moment the question, "Has the Society fulfilled the purpose for which it was originated? namely the promotion of microscopical investigation and the introduction and improvement of the Microscope?" I think we may answer confidently and emphatically *Yes*! It is not merely the instances of important inventions and improvements, such as those before referred to, and such as Mr. Stephenson's suggestion of homogeneous-immersion lenses which Prof. Abbe worked out, and the same gentleman's binocular Microscope, so admirable for dissecting purposes, etc., all of which have been the direct outcrop of the Society, but it is in an even greater degree the hundreds of small improvements which have been gradually brought out under its

influence, and have formed a constant progress towards the present excellence of the instrument; and most of all it is the steady encouragement which the Society has been able to afford over half a century to improvements in the instrument and to its intelligent use, an influence which has spread all over the civilized world and has resulted in hundreds of other societies and institutions with more or less similar objects springing up in this and foreign countries, that have formed the real value of this institution. During its more than fifty years of existence, the Microscope has grown from the comparatively rarely used and imperfect possession of a few men of science and the amusement of a few children to be the highly finished and most important companion of almost all investigators of nature; sold in thousands, and employed alike by the physician, the anatomist, the general biologist, the botanist, the mineralogist, and even the physicist, and extending its range into commerce and agriculture. There is at the present time hardly a biological or medical society in the world which does not derive a large part of the discoveries laid before it from investigations made by the aid of the microscope, and I believe that this Society and its influence have been most important factors in this great progress. There is also plenty of admirable biological work to be found in the Transactions both by the eminent men before referred to and by Allman, Caruthers, Dallinger, Drysdale, Rupert Jones, Klein, Ray Lankester, Murie, and others.

Finally, I will say a word as to the future: an idea is, I think, prevalent in some quarters that the Microscope is now perfect and that consequently the chief *raison d'être* of this Society is over. I am not by any means one of those who take this view; we may not for the moment see how further improvements are to be made, but people rarely do until they are made. There is a tendency in the human mind when a considerable progress has been made either in invention or investigation to say, "Now we have got to the end of it, there is not any more to come"; but this seldom turns out to be correct; it has been said about the Microscope over and over again as each step was made. I will only quote one instance: there were few of our early microscopists who knew more about the microscope or contributed more to its improvement than Dr. Goring; in his exordium to the first edition of his "Microscopic Illustrations," published in 1829, the learned doctor says, "Microscopes are now placed completely on a level with telescopes and like them must remain stationary in their construction." In January, 1830, Lister published his era-making paper on improvements in the Microscope before referred to.

II.—THE CYTOPLASMIC AND NUCLEAR INCLUSIONS ASSOCIATED WITH SEVERE ETCH VIRUS.

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Three Plates.

Introduction.

Although over 100 plant virus diseases have been described, in less than one-fifth of these have abnormal inclusions been described. No doubt they occur in some others, but in many they have not been found, although an extensive search has been made. This is in striking contrast to virus diseases of animals, in almost all of which inclusions have been found. In the animal diseases inclusions in the nuclei are as common as those in the cytoplasm. In plant diseases, on the other hand, there had been no convincing demonstration of intranuclear inclusions until recently, when Kassanis (1939) found intranuclear crystals to be a constant symptom of infection with severe etch virus. Kassanis compared these in size, stability, and staining reactions with the intranuclear inclusions of the polyhedral disease of silkworms. He also found many cytoplasmic inclusions of the same type as the amorphous bodies produced by some strains of tobacco mosaic and some other plant viruses. This paper describes some further properties of the two kinds of inclusions induced by severe etch virus.

Material and Methods.

Most of the work was done with *Nicotiana tabacum* var. White Burley, but *N. glutinosa* and *Hyoscyamus niger* were also used.

Living tissues were usually examined by transmitted light, but occasionally dark ground illumination or polarized light was used.

Micrurgical methods, similar to those employed in the examination of some of the inclusions produced by strains of tobacco mosaic virus (Sheffield, 1939), were used.

Microtome sections of many different tissues were examined. The fixative used for most of these was Champy's fluid, which was known to give good results with these hosts (Sheffield, 1933). Kassanis (1939) stated that the plate-like crystals were destroyed by acetic acid, so fixatives containing this were at first avoided. It was later found to be true of only very high con-

centrations of acid, and then Bouin's fixative and also Allen's modification were used with good results, but the chondriome was, of course, not preserved. Formol-saline as suggested by Kassanis gave only poor results when material was fixed in bulk. It was found to be a little more successful but not very good for epidermal strippings. As Kassanis found, Kull's staining method gave very spectacular results, and this was used for many of the preparations. In attempts to differentiate between the various normal and abnormal cell constituents many staining combinations were used. These are detailed in Table III (p. 39).

Attention might be drawn to one of them. Difficulty is often experienced in differentiating between the nucleus and cytoplasmic inclusion bodies. Feulgen's reagent with a counterstain is often used for this purpose, as the nucleus invariably stains with leuco-basic fuchsin whereas all the virus inclusions in plants yet tested fail to do so. Carbol fuchsin (basic fuchsin in phenol) differentiated in picric acid in clove oil provides a similar but further differential stain for some bodies. It was first used for plants infected with severe etch virus when the nucleus stained red and the amorphous inclusions yellow. Similar results were obtained with hosts infected with aucuba and tobacco mosaic and with Hy. III virus. Actually it is not as good for severe etch material as some others, for the red of the chromatin tends to be rather diffuse and to obscure the pale yellow of the intranuclear inclusions.

Description.

Morphology.—The amorphous bodies look like those induced by aucuba mosaic or Hyoscyamus III viruses. When first formed they are diffuse but later become more compact. Generally they are larger than the inclusions of aucuba mosaic and are more often ovoid than spherical (Pl. I, figs. 2–3). This is probably often a sequel to their large size, the width of the cell being insufficient to contain them if they were spherical. Soon after infection small granules appear in the cytoplasm, and the bodies are built up by these coalescing in the same way as the amorphous bodies produced by aucuba mosaic or Hy. III viruses. Usually they do not become vacuolate; when they do it is at a late stage preparatory to dissolution (Pl. I, fig. 6). They are less homogeneous than the aucuba mosaic bodies and obviously consist of large numbers of minute particles, many of which are doubly refractive when viewed between crossed Nicol prisms. The bodies are more stable than those of aucuba mosaic, which are immediately destroyed by pressure on the cell wall in the region of the body (Sheffield, 1939). Such treatment causes the dispersal of the particles composing the severe etch body but not their dissolution (Pl. III, fig. 5). The aucuba mosaic inclusion is immediately destroyed by pricking. Within a few seconds it either disappears or else a hyaline vesicle is formed which disappears in the course of a few minutes. By contrast a microneedle can be thrust into or through a severe etch inclusion with no apparent effect (Pl. III, fig. 3). By micromanipula-

tion such a body can be divided into portions, each of which will then persist unchanged (Pl. III, fig. 4).

The intranuclear inclusions take the form of rectangular (often square) plates (Pl. I, figs. 3 and 7 and Pl. II, fig. 4). When first formed their sides may be as little as 1μ in length (Pl. I, fig. 7). They increase in size, and their increase would seem to be analogous to the growth of crystals in a saturated solution. Some grow until they can only just be accommodated within the nucleus, their sides then measuring about 10μ. They are extremely thin, the largest being less than $0{\cdot}5\mu$ in thickness. Because they are thin a large number can be packed within a single nucleus. The nucleus of a very young cell may contain only a single small crystal (Pl. I, fig. 7), but in adult cells several usually occur together (Pl. I, figs. 2–5). Kassanis (1939) has described 15 within one nucleus, and it is difficult to count a greater number than this by direct observation of the nucleus within the cell. In nuclei that were withdrawn from adult cells by a micro-needle and then either torn to pieces or immersed in water until the nucleus burst open, however, more than 30 crystals were often found in one nucleus. The crystals withstand much maltreatment. They can with difficulty be crushed by pressure from the tip of a microneedle, when they leave a shapeless mass.

Mode of Formation.—The time of the first appearance of inclusions varies with the age of the plant and with the conditions of growth. Under poor light conditions and at low temperatures the production of inclusions, as of many symptoms, may be inhibited or considerably delayed. The intranuclear inclusions always appear before the cytoplasmic ones. In the summer rubbed leaves usually show yellow lesions within five days, and on the sixth day the veins of younger leaves become cleared. At this time no abnormalities can be seen, either in the nuclei or in the cytoplasm. On the following day as many as a dozen crystals may be counted in many of the nuclei. At the same time, the cytoplasm is seen to be very conspicuous and its streaming greatly accelerated, and minute, highly refractive particles can be seen in it (Pl. I, fig. 1). These particles are carried in the cytoplasmic stream, and when they meet they fuse and large masses are quickly built up. The process is often much more rapid than with aucuba mosaic virus. Single large masses may be produced within two days of the appearance of external symptoms. These become slightly more compact and usually assume an ovoid form, although the surface often remains irregular. Cells of ***Hyoscyamus niger*** infected with severe etch virus behave similarly to those infected with aucuba mosaic virus, the inclusions often remaining as a number of diffuse masses spread throughout the cell.

Kassanis (1939) had found both cytoplasmic and intranuclear inclusions to be absent from the growing points. It seemed possible that, if the development of the cell were followed from the meristematic stage until it reached maturity, some information as to the origin of the intranuclear inclusions might be derived.

In the leaf of the healthy tobacco plant the cells remain meristematic


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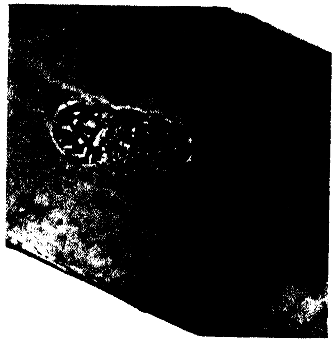
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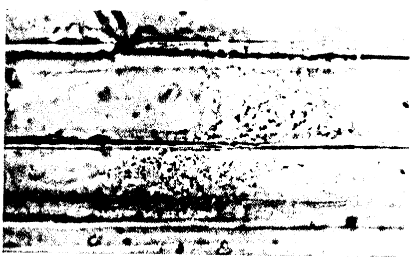
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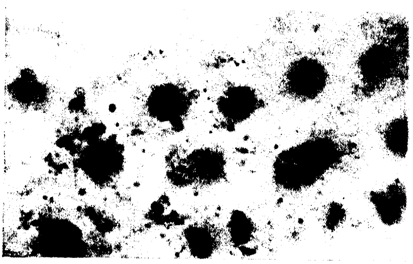
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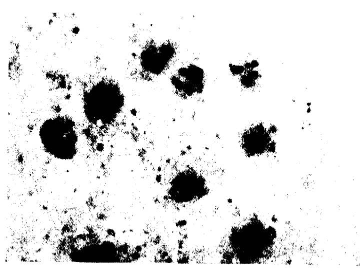
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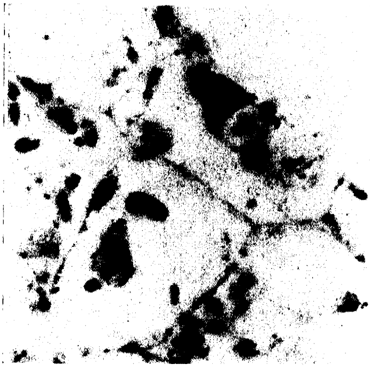

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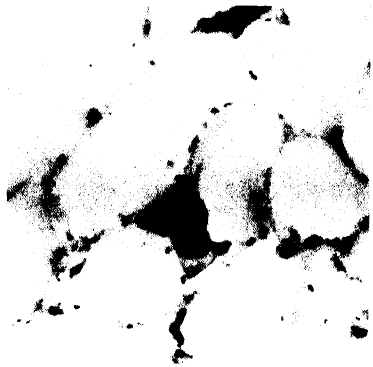

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6a

6b

only until the leaf is about 3 mm. in length. After this no nuclear or cell division occurs and growth is due to increase in size and separation of the cells. When meristematic, the cells are small and closely packed. Each contains a nucleus which occupies a large portion of the centre of the cell. It is surrounded by viscous cytoplasm with numerous tiny vacuoles. By appropriate methods chondriosomes and proplastids can be demonstrated in the cytoplasm. When mitosis and cytokinesis cease, the cells enlarge and assume the form appropriate to their function. The nuclei do not enlarge, nor does the amount of cytoplasm appear to increase. The vacuoles increase in size and join together until thin layers of cytoplasm line the walls and surround the nucleus and a few strands cross the vacuoles. At the same time in certain cells the plastid primordia develop gradually into plastids.

In plants infected with severe etch virus the meristematic growth of the young stem and leaves appears to be quite normal. After the telophase of the last division, when the nuclear membrane is reformed and the chromosomes commence to lose their identity, several small nucleoli appear in close contact with certain of the chromosomes. As the nucleus passes into the resting stage these nucleoli fuse together to form usually a single large body, but occasionally two, or, more rarely, three. It is when the nucleoli are appearing in the reconstituted nucleus following the last meristematic division that the first cytological abnormalities are observed in infected plants. At the same time small plate-like bodies appear in the nuclei and rapidly increase in size and number. They appear to crystallize from the cell sap and are not formed in contact with chromosomes, as are nucleoli.

In many of their reactions the crystals behave similarly to the nucleoli. It therefore seemed possible, especially as the former appear almost immediately after the latter, that the intranuclear inclusions were formed at the expense of the nucleoli. But the production of crystals does not result in any diminution in the number or size of the nucleoli, and in adult cells containing many large crystals the nuclear structure seems to be otherwise exactly as in the cell of the healthy plant. It then seemed possible that infection resulted in an excessive production of nucleolar material, but their staining reactions (Table III) show that although the two substances are similar they are not identical.

Besides inducing crystal formation, infection may also induce further mitoses. These usually follow rapidly on what would normally be the final cell division but sometimes occur in cells already differentiated. When, after the prophase, the nuclear membrane disappears the crystals are then thrown out into the cytoplasm. Apart from lagging of some of the chromosomes at anaphase the division appears to be quite normal until the telophase. Then the daughter nuclei are reconstituted in the usual way but no cell plate is formed across the centre of the spindle and cell division does not occur. A similar karyokinesis which is not followed by cytokinesis occurs in *Nicotiana glutinosa,* when necrotic lesions are formed as a result of rubbing the leaf surface with suspensions of strains of tobacco mosaic virus (Sheffield,

1936). Such divisions result in the production of binucleate cells. As soon as the daughter nuclei are reconstituted, further crystals appear in them. The crystals released from the parent nucleus remain for a time in the cytoplasm. It is not known whether they later dissolve, but such bodies were found only occasionally in adult cells. If all persist it would be expected that they would be observed more frequently.

In some respects these nuclear inclusions seem comparable to the protein crystals which occur regularly in the nuclei of certain healthy plants. In the case of Rivina, Stock (1892, *cf.* Tischler, 1934) was able to induce the production of similar but extranuclear crystals by giving excessive nitrogen. Tobacco plants were therefore given a weekly top dressing of 0·6 gm. $NaNO_3$. In some the dressings began before inoculation, in others at the time of, and in others, after infection. In no case was the formation of extranuclear inclusions induced. In some cases the treatment caused the cytoplasm to become increasingly conspicuous.

Distribution.—Inclusion bodies are often confined to certain tissues. Those of aucuba mosaic are most prevalent in the tegumentary tissues (Sheffield, 1931). They occasionally occur in the palisade parenchyma but rarely in other tissues. They are not found in the meristem. Those of tobacco mosaic are more widespread, and occur even in actively growing tissue (Goldstein, 1926), as also do those of mosaic-infected dahlia plants (Goldstein, 1927). Those induced by Hy. III virus which bear many similarities to severe etch are relatively numerous and occur in many tissues. This similarity extends to the amorphous inclusions caused by severe etch virus which are numerous in all tissues. Often the cells containing them do not attain their full size. As they are usually contained by almost every cell over large areas of tissue, an uneven growth follows, resulting in deformity of individual leaves and stunting of the plant.

The intranuclear inclusions of severe etch virus are even more widespread than any other virus inclusions so far recorded. The intranuclear inclusions in animals infected with viruses are usually confined to certain organs but those of severe etch occur in almost every tissue and are contained by most of the cells. Both types of inclusion are rare in very old tissues but are abundant in all those tissues which were in an actively growing condition at the time of inoculation or which were produced after infection. Neither type occurs in the tissue which is normally meristematic, although intranuclear inclusions are found in cells where mitosis is abnormally induced. Both types occur in tegumentary tissues, including the hairs, epidermal cells, and guard cells of the stomata, and in the assimilating tissue and general ground tissues of the leaf, stem, and root. In the vascular bundles they have not been found in the xylem vessels but are prevalent in xylem parenchyma and phloem. In the sepals and petals both types occur as frequently as in the leaves. In all these organs intranuclear inclusions are found in almost every cell and amorphous inclusions in many of them.

Because the inclusions of severe etch were found to be so widespread a

special examination was made of the reproductive organs. Inclusions of both types occur frequently in the filaments and more rarely in the anther walls, but none was observed in the tapetum. Neither do they appear to be formed in the pollen-mother cells, nor in the pollen grains either before or after nuclear division. They are abundant in the ovary wall, also in the style, but although they occur just below the stigma they were not found in the cells composing it. They could not be found in young ovules, either in the nucellus or embryo-sac. In the seed they were not found in the endosperm or in the young embryo. Nuclear, but not cytoplasmic, inclusions were of frequent occurrence in the seed coat.

Although inclusions were not found in other parts of the seed, their presence in the testa suggested a possibility of the presence of virus in other parts of the seed. Tests for seed transmission of the virus were therefore made. Seeds from infected tobaccos were sown immediately on ripening. None of the 408 seedlings obtained showed any virus symptoms, and inoculations made from them to healthy tobaccos all gave negative results. 198 seedlings obtained from seed of infected *Hyoscyamus niger* were also healthy.

Seed taken at the time of ripening, crushed, diluted to 1 in 10, and inoculated to healthy tobaccos gave no infections. The virus did not appear to be inactivated by other seed contents as crude infective juice diluted with seed extract gave as many infections as juice diluted with water. As the virus is rapidly destroyed by desiccation it seemed more probable that it was inactivated by the drying out which occurs in the natural course of ripening of the seed. This was confirmed when unripe seeds taken from infected tobaccos, crushed, and inoculated to healthy plants gave infections.

Persistence.—Most writers on inclusion bodies have been concerned mainly with their morphology and chemical reactions. Little has been written about their formation and persistence. It is known that the inclusions of some viruses disappear after an interval. Neither the amœboid bodies of tobacco mosaic nor the striate material are found in very old plants. The amorphous inclusions of aucuba mosaic crystallize after a few days or weeks, and later the crystals dissolve. The inclusions of Hy. III virus disappear sometimes, giving rise to needle-like bodies.

With severe etch virus both types of inclusion body disappear from the oldest leaves, but they continue to be produced in the young tissues of tobacco plants even eighteen months after infection.

Needle-like bodies may be formed within the cytoplasmic inclusions in plants infected with severe etch virus. In the majority of leaves they are of rare occurrence but in certain types occur regularly. Severe etch causes the deformation of some leaves, the laminæ being misshapen or reduced to extremely narrow strips along each side of the midribs. In other leaves which are of normal shape the sticky secretion normal to the tobacco plant is greatly reduced, so that the leaves appear almost glabrous. In these types the cytoplasmic inclusions almost invariably contain the needle-like bodies.

The needles may be from 1–5μ in length and are extremely narrow (Pl. I, figs. 4–6). When viewed between crossed Nicol prisms they are seen to be doubly refractive. In older leaves inclusions containing these needles also contain numerous smaller birefringent particles which show active Brownian movement within the amorphous inclusions, suggesting a more liquid phase containing numerous particles, many of which are needle-like. Later these bodies become vacuolate, and the needles are then seen in the reticulum so formed (Pl. I, fig. 6). This is thought to be a stage in the complete dissolution of the amorphous inclusion body.

Virus Content of the Amorphous Inclusions.—Cells were mounted in 0·1M phosphate buffer at *p*H 7 and their amorphous inclusions extracted from them.* The inclusions were then suspended in water, in which they broke up, and stored until a sufficient number were collected. So far as was possible, roughly spherical inclusions of about 20μ diameter were chosen. When 20 were collected the suspension was diluted to 1·6 c.c. Assuming their specific gravity to be about unity, this gave a concentration of approximately 1 gm. in 2×10^7 c.c. 0·3 c.c. of this was further diluted to 1·5 c.c. to give a dilution of 1 in 10^8. These dilutions were then rubbed over the leaves of young tobacco plants and compared for infectivity with a $\frac{1}{200}$, and a $\frac{1}{1000}$ dilution of crude infective sap. A water control was also included. After an appropriate interval (determined afresh for each experiment by inoculating plants on the day prior to the main experiment and making daily tests on the leaves) leaves were tested for starch lesions. This experiment was repeated three times. The results are summarized in Table I.

TABLE I.

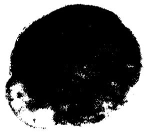
Infectivity of Amorphous Inclusions of Severe Etch Virus.

Inoculum.	Total numbers of starch lesions.
Water	0
Inclusions at dilution of 1 in 10^8	27
,, ,, ,, 1 in $2 \cdot 10^7$	91
Crude juice at dilution of 1 in 10^3	29
,, ,, ,, 1 in $2 \cdot 10^2$	147

Comparisons were also made of the infectivity of the inclusion body and of the remainder of the cell. Halves of cells containing inclusions were isolated. Also, those halves of inclusion-containing cells which were devoid of inclusions were collected. 20 half-cells of each series were suspended in 1·6 c.c. Comparisons were made with a water control and also with a 1 in $2 \cdot 10^2$ dilution of crude infected sap. The results of two experiments are shown in Table II.

* Method as described by Sheffield, 1939.

1a



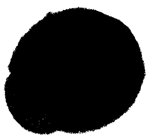
2a

3

1b

2b

1c



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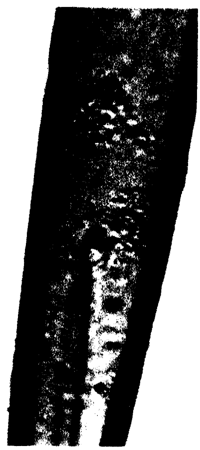

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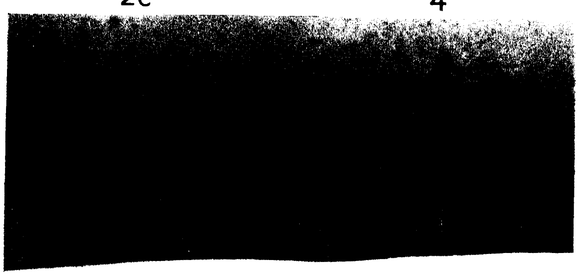
1d



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[*To face p.* 36.

TABLE II.

Inoculum.	Total numbers of starch lesions.
Water	2
Half-cells with inclusions	19
Half-cells devoid of inclusions	12
Crude juice at dilution of 1 in $2{\cdot}12^2$	72

No tests were made on the infectivity of the intranuclear inclusions. It was not difficult to isolate them, either separately or in groups as contained within the nuclei, but no suitable method of dissolving them is known. The known solvents would inactivate any virus which might be present. They can be broken mechanically but only if handled individually. They are too small for there to be any certainty of breaking them if handled in mass. Until a suitable method of suspension is found it would be unwise to attempt to test their infectivity.

Chemical Properties and Staining Reactions.—Like the amorphous inclusions of aucuba mosaic (Sheffield, 1933), those of severe etch virus obviously contain mixtures of different substances. By various techniques of fixing and staining the presence of chondriosomes and oil globules can be demonstrated, the latter appearing to be rather more abundant in the severe etch inclusions than in those of aucuba mosaic. Unlike the bodies of aucuba mosaic, those of severe etch contain particles which are doubly refractive when seen in polarized light. The intranuclear inclusions are in the main homogeneous. By a technique such as Kolachev's osmic impregnation method occasional small dark staining particles may be found within them (Pl. II, fig. 4). Careful examination between crossed Nicol prisms shows these plate-like bodies to be doubly refractive when seen in edge view but not when viewed flat (Pl. II, figs. 6*a* and 6*b*).

In strong acids such as hydrochloric, the intranuclear inclusions are soluble, and also in strong alkalis such as 10 p.c. caustic potash. The amorphous bodies are unaffected by strong acids but part of their substance is soluble in alkali. More delicate tests on the effects of slight amounts of acids and alkalis were made by removing the inclusions from the cells and suspending them in solutions of known *p*H. In the case of the amorphous inclusions these had to be of an osmotic pressure not less than that of the cell sap, for lower pressures cause dissolution of the bodies. The intranuclear inclusions persist unchanged for many hours after suspension in distilled water. They are unaffected over a *p*H range from 10 to 2. The amorphous inclusions of both aucuba mosaic and severe etch viruses are unaffected by increasing acidity (Pl. III, figs. 2 *a–c*), but at *p*H 10 a large portion of the body is soluble. Bodies suspended in such solutions rapidly shrink to a fraction of their original size. At *p*H 8 the shrinkage is more gradual. Some portion of the aucuba mosaic inclusions appears to be soluble in weak alkalis. If a body is removed into a phosphate buffer solution at *p*H 8 and 0·1 molarity, it

appears first to swell, a portion of the contents seems to liquefy. Then shrinkage occurs, the amount of solid matter remaining unchanged but the liquid seeming to be withdrawn (Pl. III, figs. 1*a–d*). The shrinkage of these bodies seems to be less than that of the severe etch inclusions.

Neither type of severe etch inclusion is affected by alcohol and both are preserved by Carnoy's fluid, which contains absolute alcohol, chloroform and glacial acetic acid in the ratio of 2 : 3 : 1.

By ether the intranuclear inclusions are unaffected but part of the amorphous inclusion is soluble. The reactions with Sudan III and Scharlach R and osmic acid suggest the presence of fats in the cytoplasmic but not in the nuclear inclusion. Both types give positive reactions with Millon's reagent and with the xanthoproteic test, suggesting the presence of large quantities of protein in each type of inclusion body. As with other plant virus bodies Feulgen's reagent drew a sharp contrast between the protein of the inclusion bodies and that of the nucleo-proteins normally occurring in the nucleus. Neither type of inclusion restored the colour to leuco-basic fuchsin but the chromatin assumed a purplish-red colour.

As the nucleolus and the intranuclear inclusions behave similarly with many reagents it seemed possible that some relationship exists between them. The behaviour of the small nucleoli in young nuclei in which crystals are forming and the presence of large nucleoli in nuclei containing up to thirty inclusions, precludes the possibility of the inclusions being formed at the expense of the nucleoli. The possibility that infection induces an excessive production of nucleolar material which takes a crystal form was tested by the trial of many staining reactions. The results of these tests are summarized in Table III.

The reactions of the amorphous inclusions vary according to the method of fixation employed. A matrix of similar but deeper staining properties to the cytoplasm was often discerned but the presence of contrasting particles within depended on the technique employed. Stains for the chondriome were used only after appropriate fixing methods, although staining techniques which destroy chondriosomes were employed after both the less and the more acid fixatives. With this explanation it was not thought necessary to tabulate also the fixatives. The intranuclear inclusions were preserved by and behaved similarly after all those employed.

It should also be remembered that any particular structure may vary in its staining capacity according to the method and degree of differentiation required by the staining combination in use. With acid fuchsin, for example, the nucleolus stains red, but washing with water removes the stain, the nucleolus becoming colourless whilst the crystals are still red. With Kull's combination the result is similar. When the same dye is used with crystal violet, differentiation is in picric acid and the red colour is allowed to remain in the nucleoli; but when in combination with methyl green, the acid fuchsin is removed and the crystals and nucleolus allowed to take the green stain.

TABLE III.—STAINING REACTIONS.

Dyes.			Reactions.				
Basic.	Acid.	Name or author of technique.	Chromatin.	Nucleolus.	Nuclear Inclusion.	Cytoplasm.	Cytoplasmic Inclusion.
Leuco-basic fuchsin (after hydrolysis) *	—	Feulgen	Red	Colourless	Colourless	Colourless	Colourless
Ditto	Orange G.	Feulgen with counterstain	Red	Orange	Orange	Yellow	Orange
Ditto	Picric acid	Ditto	Red	Yellow	Yellow	Colourless	Yellow
Ditto	Light green	Ditto	Red	Green	Green	Pale green	Green
Ditto	Light green (mordant and differentiate in Na_2CO_3 solution). †	Semmens and Bhaduri (1939)	Red	Green	Green	Colourless	Green
Basic fuchsin in phenol (after 1 p.c. HCl)	Picric acid in clove oil (used to differentiate basic stain)	Ziehl modified by Lenoir (1932)	Pink reticulum Bluish-red chromosomes	Red	Yellow	Colourless-yellow	Yellow
—	Acid fuchsin	—	Red	Colourless-red	Red	Colourless	Pink
Methyl green	Ditto	Guignard (*cf.* Bolles Lee)	Reticulum pink Chromosomes green	Green	Green (pale)	Pink (pale)	Pink and green granules
Crystal violet	Ditto (differentiate in picric acid)	Nebel (*cf.* Bolles Lee)	Purple	Red	Yellow-red	Red	Purple and red granules
—	Methyl-blue, Eosin } Mixed	Mann	Blue overlays red	Red	Red	Red	Red
Methyl green, Pyronin B } mixed (differentiated in resorcin)	—	Pappenheim	Green overlays red	Green overlays red	Green	Colourless-pale green	Red and green granules
Pyronin B (differentiate in resorcin)	—	—	Red	Red	Colourless	Colourless	Red granules
Magdala red	Light green	—	Red	Colourless-green	Colourless-green	Pale green	Red
Thionin in phenol	Orange G in absolute alcohol (used to differentiate basic stain)	Stoughton	Blue	Purple	Purple	Orange	Purple-blue
Toluidine blue	Acid fuchsin (in aniline oil water) Aurantia	Kull	Blue	Red	Red (more intense than nucleolus)	Chondriosomes, etc., red in matrix of yellow-green	Red granules and globules in matrix of yellow-green
Hæmatoxylin (mordant and differentiate in iron alum)	—	Heidenhain	Black	Colourless-black	Colourless-black	Colourless with dark staining chondriosomes, etc.	Chondriosomes and other granules and globules black in colourless matrix
Gentian violet (mordant and differentiate in iodine in alcoholic KI)	—	Newton	Purple	Colourless-purple	Colourless-purple	Colourless	Purple granules
Gentian violet (mordant 2 p.c. aq. tannin)	—	Chen	Purple	Colourless-purple	Colourless-purple	Colourless	Purple granules
—	Osmic acid (impregnation method, 7 days at 40° C.)	Kolachev	Black	Brown	Brown	Colourless with black chondriosomes, etc.	Colourless matrix containing very many black particles

* As the usual method of hydrolysis for 4 min. at 60° C. caused shrinkage in the nuclei, this method was modified, hydrolysis being carried out at room temperature over a longer period (about 1 hour).

† The results given by this method were better defined than when the light green was used as a simple counterstain.

The nucleolus and the nuclear inclusions reacted similarly to many of the stains used. However, with Pappenheim's stain the nucleolus seemed to be red and overlaid with green, whilst the inclusion appeared to have taken the green dye only. Preparations single-stained with pyronin B showed red nucleoli, whilst the inclusions were left colourless. It was later found possible to differentiate between them by the use of carbol fuchsin differentiated with picric acid. The nucleolus then took a red stain whilst the inclusion became yellow. The nucleolus and the crystal also responded somewhat differently to acid fuchsin by whatever method it was applied or differentiated.

Discussion.

The Cytoplasmic Inclusions.—The amorphous inclusions of severe etch show many points of similarity with those of aucuba mosaic virus (Bawden and Sheffield, 1939). Their mode of formation by the aggregation of particles appears to be identical. Both are obviously mixtures of chemically different materials. Each contains a large proportion of a protein constituent, and chondriosomes and fats are present in both, but the severe etch bodies appear to contain a rather larger proportion of fats. Both disintegrate if suspended in media of lower osmotic pressure than that of the cells from which they are extracted. Both contain virus, but in both cases virus is present also in the rest of the cell.

The numbers of lesions given by a 1 in 10^8 or 1 in $2 \cdot 10^7$ dilution of aucuba mosaic bodies is comparable to the numbers of lesions given by similar dilutions of purified virus. But with severe etch virus few infections can be obtained with expressed sap at a dilution greater than 1 in 10^3, but a suspension of inclusions gives infections even when diluted to 1 in 10^8. The amount of virus contained in expressed sap of hosts infected with severe etch is many times less than that obtained if the infection is with aucuba mosaic. But the results obtained with the inclusions suggest that comparable weights of the two viruses may produce comparable amounts of infection. It may not be that less virus is produced in hosts infected with severe etch but possibly it is in such a form that it is not so readily available.

As the inclusions are infective they must contain virus, and as they do not give Feulgen's reaction it seems that if severe etch virus is a nucleoprotein, like the other viruses isolated, it must also contain nucleic acid of the yeast type. Unfortunately there is no simple colour test known for nucleic acid of this type.

Bodies induced by aucuba mosaic are isotropic whereas those of severe etch virus contain minute doubly refractive particles which in some cases are in active Brownian movement within the body. The inclusions of both viruses tend to crystallize. The aucuba mosaic bodies usually give hexagonal crystals, but sometimes give needle-like forms of 20 or more microns in length. The severe etch bodies may give birefringent needles which are smaller, being only 2–5μ in length. Both types of inclusion tend to disappear from older

cells. Both may become vacuolate, the aucuba mosaic body soon after formation but the severe etch body only in the process of dissolution.

The severe etch bodies are less compact than those of aucuba mosaic, which behave as if they are bounded by a surface membrane. This is suggested by their appearance, by their behaviour in weak alkalis, where swelling occurs prior to shrinkage, and in their behaviour on pricking, when a vesicle may be formed prior to complete dissolution. The severe etch bodies can be pricked or divided without any apparent effect. In this they are similar to the bodies of Hy. III virus, to which severe etch shows many affinities. The amorphous bodies of the L strain of potato virus X, which take an amœboid rather than a granular form, can be similarly divided into portions without causing general disintegration.

As to the nature of the amorphous bodies of severe etch virus, it is at present only possible to reiterate the suggestion made as to the nature of the aucuba mosaic bodies, i.e. that the protein constituent consists of virus possibly in combination with some other cell constituent either normally present or produced as a result of infection. Differences between the physical properties of the bodies of the two diseases might be accounted for by those slight chemical differences in the structure of the viruses which confer on them their differing properties. The relatively larger number of bodies produced by severe etch virus might account in part for the apparent lower concentration in expressed sap. Virus in the inclusions would be less readily extracted than that which may be suspended in the cell sap.

The Nuclear Inclusions.—In considering these inclusions two questions immediately spring to mind. Firstly, what is the relationship between these bodies and other structures normally present in the cell, especially those within the nucleus? Secondly, what is the relationship between the crystals and the virus?

The foregoing experiments have shown that although both contain protein the chromosomes are basophilic in their staining reactions whilst the crystals are acidophilic like the nucleoli. But they show some differences in staining capacity from the nucleoli. They are not formed in connection with or at the expense of the nucleoli.

In many ways these intranuclear inclusions seem to parallel closely the crystalline inclusions which have been described as occurring in normal plants. Tischler (1934) has listed and discussed these. His list contains more than 100 species, comprising many genera among the Pteridophyta and Gymnospermæ as well as dicotyledons and monocotyledons. None has been described among healthy plants of the Solanaceæ.

These crystals are dissolved by pepsin and give protein colour reactions. They are insoluble in water. As to their solubilities in acids, alkalis, and alcohol, there is no agreement, probably due to the existence of different solubilities in the inclusions of different species. They appear to be related to the nucleoli from which they can be distinguished by staining reactions. If preparations are stained in pure acid fuchsin and then washed in water

the dye leaves the nucleolus whilst the crystal is till red. The crystals of plants infected with severe etch behave similarly. In healthy plants a double stain of Delafield's hæmatoxylin and acid fuchsin results in purple nucleoli and red crystals. This has not yet been applied to virus-infected material. In some cases (e.g. Galtonia) large crystals accompany small nucleoli, and vice versa. In *Dahlia variabilis* the nuclei contain either a nucleolus or a crystal. One of the guard cells of a stoma may contain a crystal whilst the other has a nucleolus in its nucleus. These crystals are elongated and may project from the nucleus.

At nuclear division the crystals may dissolve within the nucleus or may pass into the cytoplasm, where they dissolve. This is similar to the behaviour of the crystals induced by severe etch virus at the abnormal divisions which may occur after their formation.

In Tischler's list *Ligustrum vulgare* is quoted as containing protein crystals in the nuclei. For comparison with the virus bodies the writer examined some species of Ligustrum. In the ordinary green varieties of *L. vulgare* and *L. ovalifolium* no crystals were found, but in a variety of golden privet they were present in every cell of the leaf. They were tetrahedral and smaller and fewer in number than those induced by severe etch virus. Their apparent absence from the green and prevalence in the golden variety suggested a possible connection between them and the occurrence of chlorosis. Especially as some variegations in privet can be transmitted by grafting (Baur, 1904, '06, '07) it is felt that the subject should be more fully investigated before further comment is made.

Intranuclear inclusions also occur in many animal tissues (Findlay, 1938). Those which accompany virus diseases have been classified according to whether they cause complete degeneration of the nucleus or whether their effect is localized. The polyhedral diseases of insects have been classified as a third group. The bodies produced by them are more angular in form and bear a certain similarity in shape and staining reactions to the severe etch bodies. But in the latter there is no apparent disturbance of the nuclear contents and the subsequent hypertrophy which occurs when inclusions are formed in the polyhedral diseases. As in plants, animal virus bodies can be differentiated from the chromatin by use of Feulgen's reagent (Ludford, 1930).

Intranuclear inclusions arise in animals from causes other than infection with virus diseases and they have been produced experimentally.

Intranuclear inclusions are seen to be fairly widespread in both plant and animal kingdoms, but no very satisfactory explanation of their occurrence has been offered. It is, of course, possible that they arise from a variety of causes. In the case of the virus bodies it is tempting to suggest that they essentially contain virus by analogy with the amorphous inclusions which have been shown to be infective. In support of this the crystals induced by severe etch virus have many staining properties in common with one component of the amorphous bodies. There is, however, no evidence that the intranuclear inclusions are infective. The polyhedra, which are the

only ones with which satisfactory results have been obtained, are definitely not infective (Glaser, 1928).

Tischler (1934) suggests that the protein crystals in plant nuclei may have an ecological significance, but he inclines to the view that they are a reserve substance. For this view there is a certain amount of support. They tend to be absent from older tissues. In one case they were present in the nucellus but dissolved later. They are present in the embryo of the ungerminated seed of Mirabilis, but as it germinates they first swell, but then fragment and dissolve. On the other hand, they are often still present in very old tissues, and in bud scales, even after shedding, and in plants which have been kept in the dark.

There is some evidence that they are due to an unbalanced metabolism. Besides their occurrence in plants infected with severe etch virus and their presence in a variegated but not in green varieties of Ligustrum, we know that in Pelargonium they may be induced by the presence of *Bacterium tumefaciens.* In Fraxinus their presence may lead to hypertrophy and subsequent degeneration of the nuclei: a process unlikely to occur in completely healthy tissue. Intranuclear crystals were found in a species of Ceratium growing in the Bay of Naples, but this same species produced no inclusions when growing in the Kiel Canal. This suggests a possible diseased condition of one group of plants. Most of the observations on intranuclear crystals in normal plants were made prior to the present century. Some of the work might with advantage be repeated, for in the light of more recent knowledge on the physiological requirements of the plant as well as on the existence and nature of diseases with an ætiological agent, it might be found that the examples cited were not in a completely normal condition. A fault might lie in their nutrition, or they might be suffering from an attack from some parasite, or they might be carrying a latent virus which showed no external symptoms. Any such causes would result in an unbalanced metabolism.

Summary.

Severe etch virus induces two types of intracellular inclusion.

The cytoplasmic inclusions are amorphous. Chemically they consist of mixtures of proteins with fats and lipoids. They are formed by the aggregation of particles which appear in the streaming cytoplasm. They may contain some birefringent particles and may give rise to small needle-like bodies. They can be pricked or divided into portions with a microneedle. They contain the virus, but this is also present in other parts of the cell. They are numerous and occur in most tissues of the plant.

The intranuclear inclusions give protein reactions and are more stable than the cytoplasmic inclusions. They take the form of thin rectangular plates, and as many as 30 may be found in a single nucleus. They can be isolated but can be broken or dissolved only with difficulty. Almost every nucleus contains them and they have been found in almost all tissues. They

occur in the seed but have not been found in the young embryo. The virus is not transmitted through the seed.

The two types of inclusion are briefly discussed and compared with other inclusions occurring in diseased and healthy tissues.

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DESCRIPTION OF PLATES.

Unless it is otherwise stated all photographs are from tobacco infected with severe etch virus.

All photographs were taken with a Leitz Makam camera. The source of illumination was either a mercury vapour lamp or a Leitz Monla lamp. Fixatives, stains, source of illumination, Wratten colour filters and magnifications are given in that order after the description of each figure.

Plate I.

Fig. 1.—Part of a hair cell from the surface of a mottled leaf. The nucleus containing several crystals lies against the wall. Small particles are seen in the cytoplasmic strands. At one junction of strands some particles are accumulating. (Living, unstained, mercury vapour, 62, ×450.)

Figs. 2 and 3.—Part of a hair cell at a later stage of infection. The nucleus contains many crystals and the cytoplasmic inclusion is fully formed. (Technique as for fig. 1.)

Fig. 4.—Part of hair cell from deformed leaf. Crystals are present in the nucleus. Minute needle-shaped bodies are apparent in the amorphous inclusion. (Living, unstained, Monla, B and 22, ×450.)

Fig. 5.—Part of epidermal cell from beneath vein of deformed leaf. Crystals are present in the nucleus. Minute needle-shaped bodies are appearing in the cytoplasmic inclusion. (Technique as fig. 4.)

Fig. 6.—As fig. 5. The cytoplasmic inclusions are becoming vacuolate. (Technique as figs. 4–5.)

Fig. 7.—Meristematic tissue of a young leaf. Several nucleoli have appeared in each nucleus. Thin rectangular crystals are also forming. Some are seen flat and others in edge view. (Champy, Kull, mercury vapour, 62, ×900.)

Fig. 8.—Very slightly later stage than fig. 7. Infection has induced a further nuclear division and one cell is seen in telophase. A crystal has been thrown out of this nucleus and is seen in edge view below the group of chromosomes on the right. Other nuclei contain many nucleoli and some contain small crystals. (Technique as fig. 7.)

PLATE II.

Fig. 1.—Nuclear division is induced in already differentiated cells. That shown in this figure was taken from the ground tissue near a vascular bundle. A crystal which was formed in the parent nucleus is seen to the left of the mitotic figure. (Champy, Kull, mercury vapour, 62, ×900.)

Fig. 2.—Nuclear division in a palisade cell has not been followed by cell division so that a cell now contains two nuclei. A crystal formed in the parent nucleus is now seen in the cytoplasm. Other nuclei contain nucleoli and also small crystals in flat and edge views. (Technique as fig. 1.)

Fig. 3.—Nucleus showing differential staining of nucleolus and crystal; the former is red, the latter colourless. (Champy, pyronin, mercury vapour, 62, ×900.)

Fig. 4.—The osmic impregnation method leaves the crystals and nucleoli brown in colour. Small dark staining particles may be included within the crystals. (Champy, Kolachev, mercury vapour, 62, ×900.)

Fig. 5.—The osmic impregnation method results in much of the material of the amorphous body taking a black stain. (Technique as fig. 4.)

Fig. 6*a*.—A strip of epidermis from beneath the leaf vein shows intranuclear and cytoplasmic inclusions. (Living, unstained, Monla, B and 22, ×450.)

Fig. 6*b*.—The same field as fig. 6*a* seen between crossed Nicol prisms. By comparison with fig. 6*a* crystals in edge view are seen to be birefringent; one which is lying flat (top, right corner) is isotropic. The amorphous bodies contain doubly refractive particles (to left and lowermost cells). Other "flares" seen in fig. 6*b* are due to secreted material lying on the leaf surface. (Living, unstained, Monla, polarized light, ×450.)

PLATE III.

Figs. 1*a*–*d*.—Inclusion of aucuba mosaic virus from *Solanum nodiflorum*. This inclusion was isolated together with the nucleus in a 0·1 molar solution of phosphate buffer at *p*H8 (fig. 1*a*). After 10 minutes it had swollen considerably (fig. 1*b*). Then the nucleus passed inside the body, which began to shrink. (Fig. 1*c* was taken 30 minutes after isolation.) After 1 hour no further shrinkage occurred (fig. 1*d*). The amorphous inclusions of severe etch virus behave similarly in alkaline solution. (Living, unstained, mercury vapour, unscreened, ×450.)

Figs. 2*a*–*c*.—Inclusions of aucuba mosaic virus from *S. nodiflorum*. Neither these inclusions nor those of severe etch show any change in weak acids. This inclusion was isolated into a phthalate buffer solution at *p*H 3·4 and 0·1 molarity (fig. 2*a*). No change was seen after 3 hours (fig. 2*b*) or 48 hours (fig. 2*c*). (Technique as fig. 1.)

Fig. 3.—The cytoplasmic inclusions of severe etch virus are unaffected by pricking. (Technique as fig. 1. The black outline is due to the hair having become partially immersed in air.)

Fig. 4.—The cytoplasmic inclusions can be divided into portions. (See fig. 3.)

Fig. 5.—If crushed the inclusions break into particles but do not dissolve. (Technique as fig. 1.)

826. 7.

III.—THE RATE OF PENETRATION OF FIXATIVES.

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Nine Text-Figures.

Introduction.

Although the times recommended in text-books of histology for the fixation of tissue pieces are usually more than sufficient to ensure complete fixation by all the ingredients of the common mixtures, the problem of the *rate of penetration* of fixatives has come to acquire an independent interest of its own. Only with regard to the great rate of penetration of acetic acid does there seem to be any agreement in the literature. This may very well be because the evidence derived from experience in the practical use of fixatives lacks a proper backing of physical or chemical theory. For instance, it is often said that fixatives *retard* their own penetration into tissues. The origin of this belief is twofold. Fixatives make tissues *look* dense and impermeable; and they are often expected to penetrate at a constant rate (Underhill, 1932), so that doubling the time of fixation should double the distance penetrated by the fixative. But the optical density of fixed tissues is due to the destruction of the fine organization of the protein system, and it is more likely than otherwise that fixatives should facilitate their own entry, just as they break down fine, incompletely permeable membranes of natural origin. The second belief, that fixatives penetrate at a constant rate, is less easy to understand—unless Maxwell's Demon should prove to be a histologist.

We shall find that fixatives penetrating into protein systems obey the laws of diffusion, so far as can be judged within the limits of observational error. They neither retard nor facilitate their own penetration. The rate of penetration of one fixative in the presence of another—except in the special instances where they interact chemically—is the same as its rate when acting separately at the same concentration in the common solvent. Fixation by mixed fixatives is therefore serial fixation by the ingredients in the order of their rates of penetration, and the rate of total fixation by the mixture is limited by the rate of the slowest. In certain instances where a fixative is used at a low concentration, the fact that it is "used up" by the material it fixes may cause a slight and calculable deviation from the normal course

of diffusion; but except in one case, *osmic acid,* this deviation is totally obscured by normal observational error. Even so, it only affects osmic acid after a considerable depth has been reached.

Experimental Methods.

In order to obtain accurate data on the *relative rates* of penetration of fixatives into tissues, and at least some idea of the *order of magnitude* of these rates, the use of actual tissue fragments is out of the question. We have therefore measured their rates of penetration into a blood plasma coagulum which, besides being homogeneous, has the same sort of chemical organization as living tissue. Plasma is prepared from the cockerel by bleeding from the carotid artery, spinning the blood, and storing the supernatant plasma in waxed test-tubes on ice. For experimental purposes it is coagulated by warming to 37° C., or more conveniently by adding a trace of a tissue extract

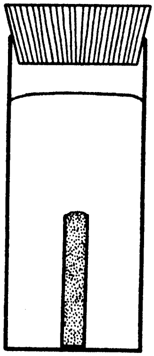

Fig. 1.

Indicators.

Potassium dichromate (neutral): *methylene blue.*
Acetic acid: *bromothymol blue.*
Osmic acid: *pyrogallic acid,* 1 p.c. solution.
Formaldehyde: *phenylhydrazine-hydrochloride*, freshly made up saturated aqueous solution.
Iodine: *starch solution.*

The proportion of indicator to plasma is 1 : 20. Methylene blue is decolorized by dichromate. The pyrogallic acid tends to use up the reagent, and to cause a calculable deviation from the normal penetration rate. Formaldehyde precipitates with phenylhydrazine-HCl. This indicator, chosen because it gives a precipitation reaction, is only just satisfactory. It retards, though it does not finally prevent, the coagulation of plasma, and causes the formation of a slight precipitate on its own account. For use with it the plasma should always be clotted with tissue extract. It should be noted that Schiff's reagent for the detection of formaldehyde may not be used in the presence of serum or tissue extract.

in saline, in short glass tubes of constant bore sealed off at one end. The actual bore of the tube, provided that it is not of capillary order, is unimportant; nor does it matter if there is a small positive or negative meniscus at the top, for the only consequence of this (see figs. 2–9) is that the regression

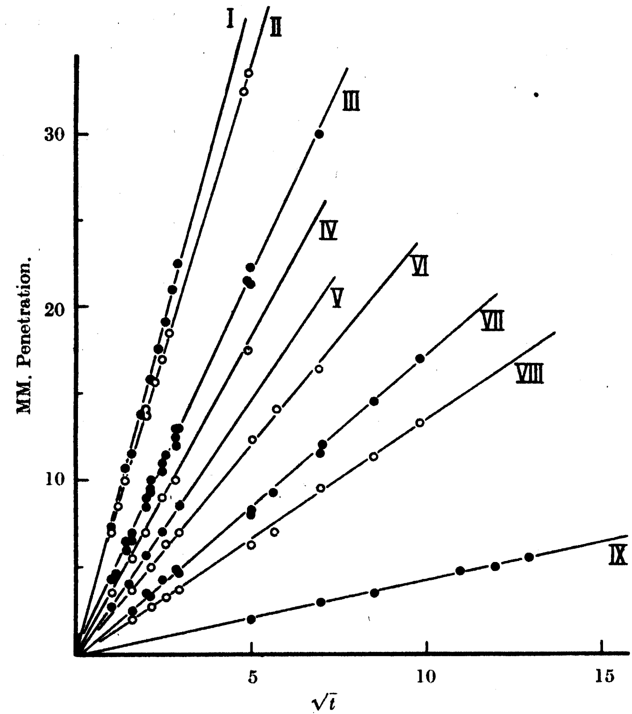

Fig. 2.

*Fig. 2.—Simple Fixatives.**

I.—*Acetic Acid* 4·75 *p.c.*, wave-front acid to bromothymol blue, K=7·809 (±0·121). This is probably a lower concentration than that at which its histological effects are exerted. During the course of the experiment a band of plasma at the top of the tube dissolves, possibly owing to the operation of plasma proteases in the acid environment, and to the dissolution of fibrin.

II.—*Formaldehyde* 40 *p.c.* (*formalin*), indicated by precipitation with phenylhydrazine-HCl. K=6·722 (±0·047). This is a higher concentration than is now normally used for fixation. Formaldehyde may take some time to exert a fixative action on proteins, and this accounts for the conflicting statements on its penetrative power in the literature. Formaldehyde *penetrates* extremely rapidly; it appears to *fix* very slowly (Underhill, 1932).

III.—*Mercuric Chloride, aqueous solution saturated at room temperature.* Fixing concentration, K=4·337 (±0·052). This is the most rapidly penetrating general protein precipitant. It fixes rather more rapidly in acid solution. (See fig. 4, II, and *Summary Table.*)

IV.—*Chromic Acid,* 1 *p.c.* solution of the anhydride, fixing concentration, K=3·599 (±0·053).

V.—*Osmic Acid* 1 *p.c.* Indicated by pyrogallic acid, K=2·977 (±0·067). Readings beyond those figured on the graph show a downward trend of the regression line for which the using up of the reagent by the indicator is responsible. Osmic acid softens the coagulum very markedly.

VI.—*Picric Acid, aqueous solution saturated at room temperature.* Fixing concentration, K=2·503 (±0·046). Below the incisive margin of precipitation there is a clear yellow band of picric acid below precipitation strength. Below this again there is a sharp margin of initial precipitation which tails off into the depths of the

* The penetration coefficients of other simple fixatives, and of these fixatives at other concentrations, will be found in the Summary Table.

lines indicating the distance of penetration do not pass through the origin. The plasma tubes are stood in stoppered vessels containing a volume of fixative, at least thirty times greater than the volume of plasma, which is periodically renewed, so that the concentration of fixative may be regarded as constant throughout the experiment (fig. 1). For protein precipitants the plasma acts as its own indicator; and with slowly diffusing precipitants, in particular, an incisive boundary is formed at the level where the reagent is of a concentration *sufficient and necessary* to precipitate the plasma proteins. The distance from the top of the tube of this fixation margin can be read with ease to the nearest 0·25 mm. For fixatives which do not precipitate proteins, like acetic acid and formaldehyde, special indicators must be incorporated into the plasma. Here arises the difficulty that the indicated reaction may not take place at the minimum fixing concentration of the reagent; but it is clear that if the concentration wave-front actually indicated obeys the diffusion law, then wave-fronts of higher or lower concentration must also do so.

The analysis of the results is not complicated. We are recording the advance into the diffusion tube of a wave-front of constant concentration. If the temperature remains steady, and the concentration of the fixative outside does not fall, then the distance (x) penetrated by the wave-front should depend upon the following simple function of the time of fixation, t:

$$x = K\sqrt{t}$$

i.e. the distance penetrated is proportional to the square root of the time of fixation. The constant K will be called the *coefficient of penetration*; in our system of units, it represents the factor by which the square root of the time in hours must be multiplied in order to arrive at a figure representing the distance penetrated in millimetres.

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tube. Picric acid, then, is below coagulation strength when it is sufficiently acid to precipitate serum globulins. These redissolve as the acidity rises, and are eventually irreversibly denatured by the picric acid. Picric acid causes marked shrinkage of the coagulum.

VII.—*Absolute Alcohol.* fixing concentration, K=1·714 (±0·023). The fixed depth separates in a remarkable way into bands of different optical density; heavy and light bands alternate. This cannot be due merely to the dehydrating action of alcohol; it almost certainly represents a "Liesegang Ring" effect which has nothing to do with the action of the fixative as such; *Carnoy* (fig. 7) shows the same effect. Alcohol penetrates curiously slowly.

VIII.—*Uranium Nitrate*, 1 *p.c.*, fixing concentration, K=1·362 (±0·027).

IX.—*Tannic Acid* 1 *p.c.*, fixing concentration, K=0·434 (±0·013). The remarkable slowness of tannic acid is a matter of considerable clinical importance (see *Discussion*). It produces a denser precipitate than any other fixative, but it neither retards its own penetration nor the penetration of other fixatives, as this experiment shows: a plasma tube was fixed for 170 hours, to a depth of about 5½ mm., with 1 p.c. tannic acid. It was then used in the ordinary way with Bouin's fixative. As soon as the Bouin penetrated past the already fixed band, the distance penetrated was found to differ insignificantly from the distance predicted from the regression line for Bouin itself (*q.v.*).

The use of gelatine tannate films as semi-permeable membranes suggests that the action of tannic acid on concentrated protein gels may be rather different. The gelatine film is itself, however, impermeable to substances of high molecular weight, and the most important consequence of tanning is that the film becomes insoluble in water.

The chief source of experimental error, which proves to be slight, lies in the variable pitch of "room temperature," at which for obvious reasons the experiments are conducted. Against this must be noted the much more important fact that the plasma coagulum is immune from the complication which normally affects experiments on diffusion—namely, the mixing of the

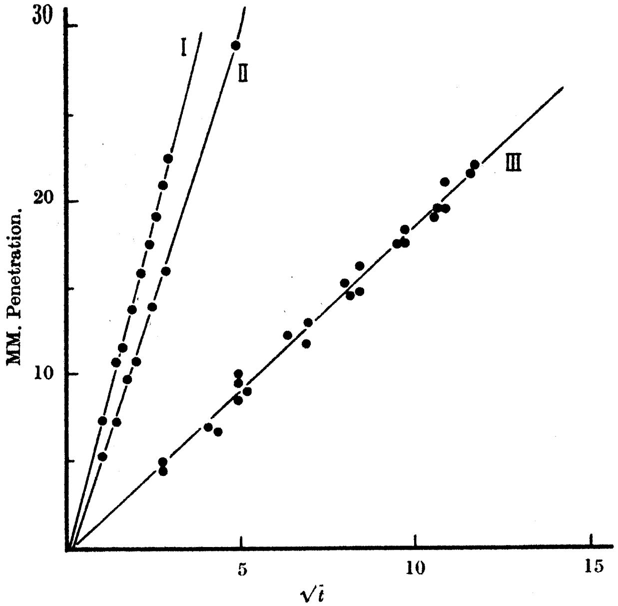

Fig. 3.

Fig. 3.—*Bouin's Fluid.*

Picric Acid, Sat. Aq. Sol.	75
Formaldehyde, 40 *p.c.*	25
Acetic Acid	5

I.—*Acetic Acid,* 4·75 *p.c.* (See fig. 2, I.)

II.—*Formaldehyde,* 9·5 *p.c.,* concentrated indicated by precipitation with phenylhydrazine-HCl, K = 6·117 (±0·095).

III.—*Picric Acid,* 75 parts of the saturated aqueous solution in 105 parts of water; and *Bouin.* Fixing concentration, K = 1·877 (±0·043). A large number of readings for whole *Bouin* and its picric acid constituent were taken in order to determine whether there is any self-retardation after 200 hours' fixation; and to show that where there is no chemical interaction the rates of penetration of a reagent in a mixture, and of the reagent treated separately, are indistinguishable. There is no self-retardation.

Picric acid shows the precipitation pattern already described (fig. 2, VI).

reagents by mechanical disturbances, counter-diffusion, and convection at their common interface.

Graphs 2–9, which together with their legends represent the experimental matter of the paper, show the distance of penetration of various simple fixatives, and of the ingredients of some common mixtures, as a linear function of the square root of the time of fixation. The data are summarized

and added to in the *Table* of penetration coefficients. Generally speaking, each *point* represents the mean of three determinations, and each regression *line* is calculated arithmetically from at least two independent sets of determinations. The fixatives themselves are freshly prepared from chemicals

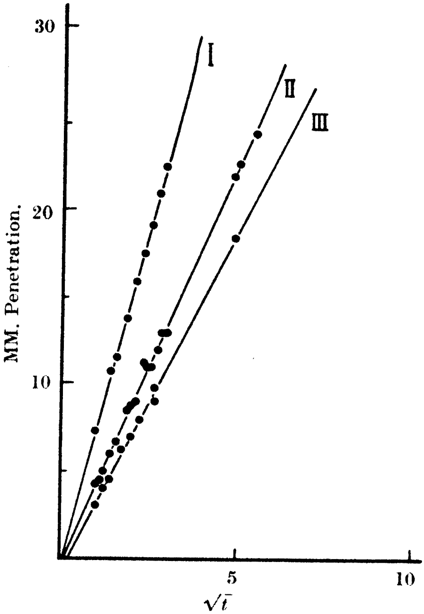

Fig. 4.

Fig.* 4.—*Zenker's Fixative.

Potassium Dichromate	2·5 g.
Mercuric Chloride	5·0 g.
Distilled Water	100·0 ml.
Glacial Acetic Acid	5·0 ml. (before use)

I.—*Acetic Acid* 4·75 *p.c.* (See fig. 2, I.) Separate tests show that the interaction of the acetic acid with potassium dichromate does not detectably affect the penetration of the acid wave-front, which is the same as that of *Bouin*.

II.—*Mercuric Chloride*, 4·75 *p.c. in* 4·75 *p.c. acetic acid.* Fixing concentration, K=4·599 (±0·060). This is faster than the unacidified saturated aqueous solution (fig. 2, III.). Thus, " corrosive acetic " is more than a mixture of fixatives.

III.—*Potassium Dichromate,* 2·4 *p.c., in* 4·75 *p.c. acetic acid.* Fixing concentration, K=3·884 (±0·049). This should be contrasted with neutral dichromate of the same concentration (fig. 9, I), which is somewhat faster (as indicated by methylene blue). The operative constituent in this fixative is presumably chromic acid, but there is an excess of acetic acid, as the notes to I show.

of reagent purity. Occasionally (as in Graph 6, *Mann's Fixative*) a trivial alteration is made in some recognized formula in order to avoid unnecessary duplication of experiments.

Discussion.

The data recorded in the experimental section give one a reliable idea of the *relative* rates of penetration of fixatives, and of their *absolute* rates of pene-

tration into a plasma coagulum. The trivial difference between the rates of penetration into the normal coagulum, one diluted with an equal volume of saline, and one of "double strength"—i.e. containing twice the physiological concentration of fibrinogen—shows that diffusion through the plasma gel proceeds at virtually the same rate as diffusion through the medium

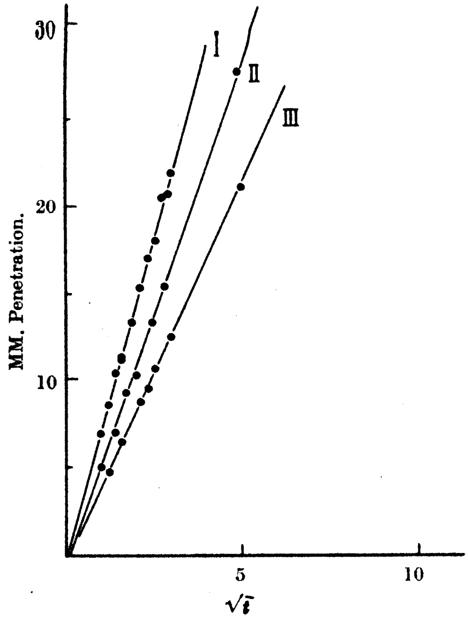

Fig. 5.

Fig. 5.—"*Susa.*"

Mercuric Chloride, Sat. Aq. Sol.	50 ml.
Trichloracetic Acid	2 g.
Formaldehyde, 40 *p.c.*	20 ml.
Glacial Acetic Acid	4 ml.
Distilled Water	30 ml.

I.—*Total Acid, and Acetic Acid* 3·9 *p.c.*, wave-front acid to bromothymol blue, K = 7·376 (±0·046). The penetration of the acid wave-front is determined by the acetic acid; the trichloracetic acid does not affect it.

II.—*Formaldehyde* 8 *p.c.* (strictly 7·7 p.c.), indicated by precipitation with phenylhydrazine-HCl, K = 5·875 (±0·077).

III.—*Mercuric Chloride.* 50 parts of the saturated aqueous solution in 104 parts of 1·92 p.c. trichloracetic acid and 3·9 p.c. acetic acid. Fixing concentration, K = 4·328 (±0·035). This is not significantly slower than the neutral saturated aqueous solution (fig. 2, III.), although it is itself only about half-saturated.

(serum) which forms its continuous phase. Fixation of tissues is, however, complicated by the presence of what may be roughly called a "disperse phase" represented by barriers of various and incalculable degrees of fineness. The use of the plasma coagulum, closely comparable to the "continuous phase" of tissues, represents the choice of the former of two alternatives: to obtain *accurate* data on the relative rates of penetration of fixatives, or

to obtain comparatively inaccurate information—about which controversy will continue to circle—using the tissue pieces themselves. At the same time it must be noted that while the data for protein precipitants are strictly sound for comparative purposes, the figures for non-protein precipitants—particularly formaldehyde—indicate the progress of a reaction without time-

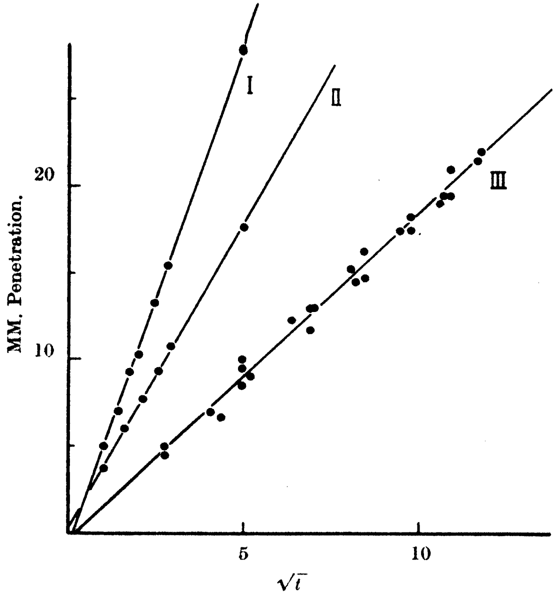

Fig. 6.

Fig. 6.—*Mann's Fixative.*

Mercuric Chloride	2·5 g.
Picric Acid, Sat. Aq. Sol.	89 ml.
Water	11 ml.
Formaldehyde	25 ml.

I.—*Formaldehyde*, 8 *p.c.* (See fig 5, II.)
II.—*Mercuric Chloride*, 2 *p.c.*, fixing concentration, K = 3·466 ($\pm$0·046).
III.—*Picric Acid*, 89 parts of the saturated aqueous solution in 125 parts of water. As fig. 3, III.

lag. It is of less importance that the reaction indicated may not occur at fixing concentration.

The concentrations and proportions of the mixed fixatives must be supposed to have been arrived at by lengthy empirical tests, though this is not by any means obvious from the literature. No useful purpose would therefore be served by proposing modified formulæ. Most of the fixatives are " well spread out " in the sense that the ingredients reach a fixed point at sharply distinct times. The nearer the point is to the outside, of course, the shorter will be the time-lag between the action of the various components. This " spreading-out " may be advantageous ; otherwise, it would be an act of economy to lower the concentrations of the faster ingredients to the

lowest concentration, compatible with fixation, which yields a penetration rate equal to or faster than the rate of the slowest.

There is no correlation between the coarseness of the grain produced by protein precipitants and their rate of penetration. The great rate of *penetration* of formaldehyde, and its apparent distinction from the rate of *fixation* (Underhill, 1932), is surprising; so also the rapidity of mercuric chloride and the slowness of absolute alcohol.

Although fixatives have a number of minor uses in surgery, their chief importance from a clinical point of view arises in the treatment of burns.

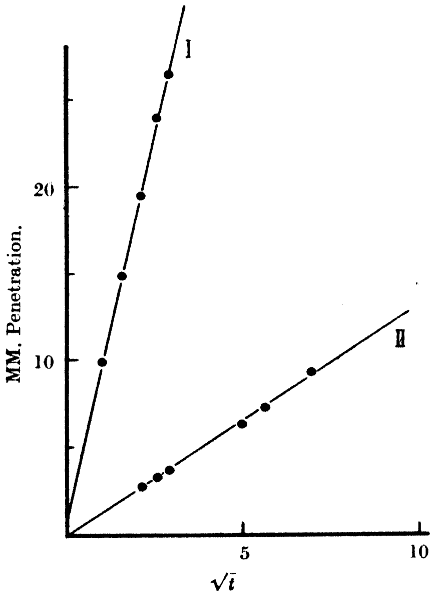

Fig. 7.

Fig. 7.—Carnoy's Fixative.

Absolute Alcohol	75
Acetic Acid	25

I.—*Acetic Acid* 25 *p.c.*v/v (in aqueous solution). Wave-front acid to bromothymo blue, K=8·816 (±0·230).

II.—*Carnoy* (Full), fixing concentration, K=1·326 (±0·035). Carnoy is the slowest penetrant of the common fixatives, and therefore only small pieces should be fixed in it. Carnoy shows the Liesegang band effect described under absolute alcohol (fig. 2, VII).

Picric acid, silver nitrate, gentian violet, and, above all, tannic acid (Davidson, 1925), are used to fix the surface of burnt areas; the relief of pain they bring about and the formation of a hard crust or tan which is fairly impermeable to exudates is a consequence of their fixative action as such. Whatever may have been the motives which originally decided the choice of these reagents, it can be seen that they are all characteristically *slow penetrants.**

* Gentian violet penetrates very slowly, but its rate has not been accurately determined.

It has been objected that the use of fixatives for the immediate treatment of severe burns may finally result in serious contractures and necrosis. It is easy to see from the figures quoted below that when these fixatives are applied by intermittent spraying or painting it is quite impossible that they should penetrate to a depth sufficient to cause fixation and atrophy of deep-lying tendons and blood-vessels. Only prolonged immersion in a bath of

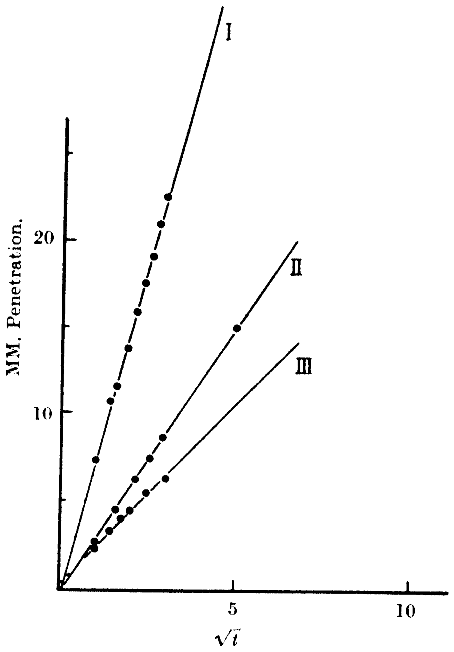

Fig. 8.

Fig. 8.—Fleming's Fluid.

Chromic Acid, 1 p.c.	15
Osmic Acid, 2 p.c.	4
Acetic Acid	1

I.—*Acetic Acid* 3·9 *p.c.* (strictly 4 p.c.). (See fig. 5, I).
II.—*Chromic Acid* 0·75 *p.c.*, fixing concentration, K=2·992 (±0·038).
III.—*Osmic Acid* 0·4 *p.c.*, indicated by pyrogallic acid, K=2·035 (±0·092). Later readings than those indicated on the graph show a deviation from the normal course of diffusion, presumably owing to the using up of the reagent by the indicator. This applies only after the ninth hour of fixation.

fixative could bring this about. It is a fact, however, that the tannic acid film conceals the extent and gravity of the lesion initially caused by a third degree burn ; and it is to this original damage that the seriousness of secondary consequences attributed to the fixatives must be due (Cohen, 1940).

SUMMARY.

1. Fixatives penetrating into a plasma coagulum—i.e. into a substance with the same general physico-chemical organization as cytoplasm and

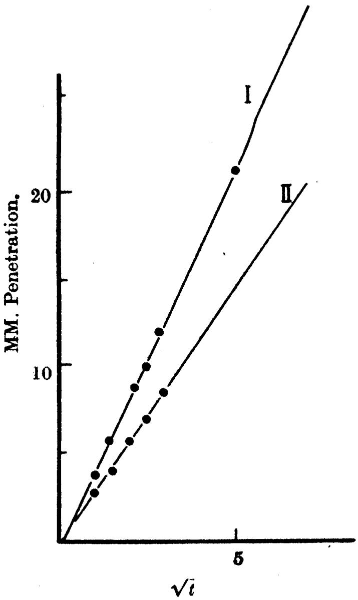

Fig. 9.

Fig. 9.—*Altmann's Fixative.*

Potassium Dichromate 5 *p.c.*	1
Osmic Acid 2 *p.c.*	1

I.—*Potassium Dichromate* 2·5 *p.c.* neutral aqueous solution. Indicated by the decolorization of methylene blue, K = 4·359 (± 0·046). By itself, neutral dichromate has no obvious effect on the plasma coagulum.

II.—*Osmic Acid,* 1 *p.c.*, indicated by pyrogallic acid. K = 2·977 (± 0·067). (See fig. 8, III.)

intercellular fluid—are shown to obey the diffusion laws within the limits of error of the experiments described.

2. The distance penetrated by fixatives is directly proportional to the square root of the time of fixation.

3. Fixatives neither retard nor facilitate their own entry into the coagulum.

4. The relative rates of penetration of a number of simple and mixed fixatives are tabulated.

I should like to thank Dr. J. R. Baker for his interest in this work and for his advice on certain technical matters.

SUMMARY TABLE.

Fixative.	Coefficient of Penetration.	Standard Error.
Acetic Acid 25 p.c.	8·816	0·230
Acetic Acid 4·75 p.c.	7·809	0·121
Acetic Acid 3·9 p.c.	7·376	0·046
Formaldehyde 40 p.c.	6·722	0·047
Formaldehyde 9·5 p.c.	6·117	0·095
Formaldehyde 8 p.c.	5·875	0·077
*Mercuric Chloride 4·75 p.c. in 4·75 p.c. acetic	4·599	0·060
Potassium Dichromate 2·5 p.c.	4·359	0·046
*Mercuric Chloride, Sat. Aq. Sol.	4·337	0·052
*Mercuric Chloride, Acid, *Susa* concentration	4·328	0·035
**Susa*		
*Potassium Dichromate 2·5 p.c. in 4·75 p.c. acetic	3·884	0·049
**Zenker*		
*Chromic Acid 1 p.c.	3·599	0·053
*Mercuric Chloride 2 p.c.	3·466	0·046
*Chromic Acid 0·75 p.c.	2·992	0·038
Osmic Acid 1 p.c.	2·977	0·067
Altmann		
Iodine, 1 p.c. in 3 p.c. KI	2·863	0·024
*Cadmium Chloride 1 p.c.	2·687	0·019
*Picric Acid, Sat. Aq. Sol.	2·503	0·046
*Cadmium Chlorate 1 p.c.	2·296	0·049
Osmic Acid 0·4 p.c.	2·035	0·092
Flemming		
*Picric Acid 71·4 p.c. of the Sat. Aq. Sol.	1·877	0·043
**Bouin*		
**Mann*		
*Silver Nitrate 2 p.c.	1·819	0·047
*Absolute Alcohol	1·714	0·023
*Uranium Nitrate 1 p.c.	1·362	0·027
**Carnoy*	1·326	0·035
*Tannic Acid 1 p.c.	0·434	0·013

Fixatives marked (*) are read at *fixing concentration.* The *penetration coefficient* is the factor by which the square root of the time in hours must be multiplied in order to arrive at a figure representing the penetration in millimetres into the standard coagulum. It should be read to *three* places of decimals, as above, when it is used as a regression coefficient; but for purely comparative purposes, when there is no cumulative error in multiplying, it should be read to the *second* place.

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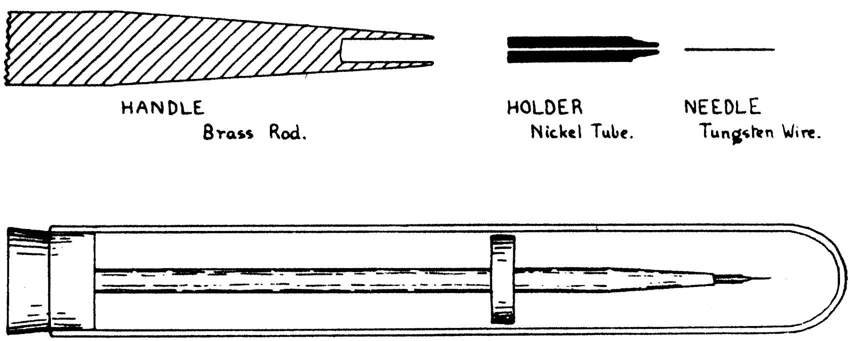
IV.—A NOTE ON FINE NEEDLES FOR DISSECTION.

By H. Graham Cannon, Sc.D., F.R.S., F.R.M.S.

One Text-Figure.

I have recently had occasion to go into the question of the making and mounting of the fine tungsten needles for Harding's micro-dissector (see Harding—this Journal, 1939, Vol. LIX, p. 19). With the valuable assistance of Dr. C. C. Paterson, of the General Electric Co., Ltd., I have standardized a method that is so satisfactory that I consider it worth publishing.

To obtain a sharp point on a tungsten needle Dr. Harding suggests either sharpening on a hone or burning the tip in a minute gas flame, but both these methods are very chancy. A perfect point can be obtained by dipping



Text-figure (1), diagram showing :—
(*above*) The three elements of the micro-needle, and
(*below*) The micro-needle mounted in a glass tube.

the end of the wire into fused sodium nitrite. This cheap chemical fuses readily over a spirit-lamp and can in fact be boiled in this heat. If now the wire be dipped into this boiling mass it at once incandesces at the extreme tip and will then be found to taper sharply to a perfect needle point. The correct time taken for this burning away depends on the thickness of the wire. The very thin wire, 0·05 mm., burns away in a fraction of a second, while wire as thick as 0·5 mm. may take a second or two.

The mounting of the needle presents the next difficulty. The method suggested by Dr. Harding of holding the tungsten wire in a rod of solder is unsatisfactory, as molten solder will not wet tungsten. To overcome this difficulty the General Electric Co., Ltd., provided me with small nickel tubes

1 mm. in diameter bearing a bore of 0·2 mm. and tapering sharply at one end. The tungsten wire is held by being inserted into the tapered end of the tube, which is then pinched with powerful pliers.

To carry this mounted needle a bore 1 mm. in diameter is drilled in the appropriate arm of the micro-dissector and the nickel tube is inserted. It can then be soldered in position, but the better method is to rely on a push fit. If it slips in and out too easily, a slight pinch with the pliers will usually make it fit into its bore quite firmly. If it is mounted in this way it can always be forcibly pulled out when it is desired to change the needle.

I have referred above to mounting the needles in the micro-dissector. Actually I find that a tungsten wire needle mounted as I have described at the end of a thin brass rod is of great value in conjunction with the micro-dissector. If the specimen to be dissected is held by the micro-dissector needle controlled by the left hand, very fine dissection can then be carried out by a similar needle mounted in a brass handle about 5 inches long. Such needles can be inserted through corks and kept in thick glass tubes on the ordinary work bench (fig. 1) without fear of damage. The cork guard near the top prevents the needle being damaged while being taken out and put back into its tube, while at the same time it forms a useful grip for the fingers while the needle is being used for dissection.

Pure tungsten wire may be obtained from Messrs. Duram, Ltd., Thanet House, 231–2, Strand, London, W.C.2.

Nickel tubes as described above may be obtained from Messrs. Johnson, Matthey and Co., Ltd., 78, Hatton Garden, London, E.C.1.

OBITUARY.

MORITZ VON ROHR.

(1868–1939.)

Prof. Moritz von Rohr was elected a Fellow of the Royal Microscopical Society in 1930. He was deeply respected by all those who knew him and his death will be regretted by a wide circle. He had contributed to the Journal on several occasions previous to his election. His writings were characterized by their profound knowledge of all branches of optics with which he dealt. He took the greatest interest in the historical side of his subject, on which his knowledge was unrivalled. At the same time his ability as a computer was of a very high order. Messrs. Carl Zeiss recognized this at an early stage, as he had been technical adviser to that firm for over forty years. In 1913 von Rohr was made Professor of Medical Optics at the University of Jena, a connection which he maintained throughout his life. His great ability in no way affected his attitude to other workers with whom he came in contact, for he had a most amiable and gentle disposition. The writer of this note last met him in Jena, after the political changes had occurred leading up to the present war. He was fully conscious of the dangers ahead but did his utmost to avoid controversy. His knowledge of English literature was greater than that of most Englishmen, and his private library was a standing tribute to his versatility. His outstanding service to practical microscopy was the computation of monochromatic quartz objectives for use with ultra-violet light. This was an achievement of the first order, as he successfully designed these objectives from fused quartz alone, the correction for spherical aberration being achieved with great accuracy by multiplication of quartz surfaces. And so we must take leave of a distinguished Fellow of the Royal Microscopical Society and an ornament to the Society in which his fellowship was a treasured possession.

J. E. B.

ABSTRACTS AND REVIEWS

BOTANY.

(*Under the Direction of* J. RAMSBOTTOM, *O.B.E.*, Dr.Sc.)

Anatomy and Morphology.

Formation of Septa in Fibre-Tracheids.—P. A. VESTAL and M. R. VESTAL (" The Formation of Septa in the Fibre-Tracheids of *Hypericum Androsaemum* L.," *Bot. Mus. Leaflets*, Harvard Univ. 8, 1940, 169–80). The development of the fibre-tracheids is normal in that they undergo elongation and maturation of the secondary wall. However, the protoplast remains active and usually undergoes division at a right angle to the original division of the cambial initial. This division consists of true karyokinesis followed by cytokinesis. The septum is formed in the region of the cell plate and the daughter nuclei migrate to the central portion of the newly formed compartments. As far as can be determined, the formation of septa is not confined to any particular region, but may occur in any part of the secondary wood formed during a single growing season. The evidence is clear that the definition of a septate fibre-tracheid suggested by the International Association of Wood Anatomists is in agreement with the observable ontogeny of the fibre-tracheid.

B. J. R.

Effects of Indolylbutyric Acid and Œstrone on the Rooting of Cuttings. N. H. GRACE (" Effects of Dusts containing Indolylbutyric Acid and Œstrone on the Rooting of Dormant *Cronicera tartarica* Cuttings," *Can. J. Res.*, 1940, **18**,c 283–8). The effect of indolylbutyric acid is to increase the number of cuttings rooted, the number and length of roots, the mean root length, and the green weight of leaf produced. Œstrone has a depressive effect and tends to offset the beneficial effects of the indolylbutyric acid, except when the two reagents are applied together in a combination of 100 p.p.m. of each, which increases root length. Results indicate that the number and length of roots and the green weight of leaf produced are more sensitive responses for demonstrating the effects of different concentrations of the acid than is the percentage of cuttings rooted. B. J. R.

Effects of Hormone Solution and Dust Treatments on the Growth of Cuttings.—N. H. GRACE and J. L. FARRAR (" Vegetative Propagation of Conifers. VI. Hormone Solution and Dust Treatments of Spruce Cuttings propagated in Greenhouse and Outside Frames," *Can. J. Res.*, 1940, **18**c, 401–14). Cuttings taken in February and April rooted better than those taken in January and March. Short cuttings gave better results than long cuttings, especially when propagated out-of-doors. Indolylbutyric acid treatment had no beneficial effect at concentrations of 20 to 60 p.p.m. Treatment with talc dusts containing indolylacetic acid, cane sugar, and organic mercury failed to show any beneficial effect, although injury from indolylacetic acid was somewhat reduced by its combination with organic

mercury. Results indicate that short Norway spruce cuttings taken from January to April root to the extent of about 50 p.c. Cuttings taken in May and June nearly all failed to root. New growth cuttings taken in late June gave some rooting, however, when propagated in sand watered with nutrient salts. B. J. R.

Effects of Naphthyl Acids and their Potassium Salts on Cuttings.—N. H. Grace ("Responses of Plant Cuttings to Treatment with Naphthyl Acids and their Potassium Salts in a Talc Carrier," *Can. J. Res.*, 1940, **18**c, 457–68). Cuttings of one herbaceous and four dormant woody plants were treated with a series of talc dust containing indolylacetic acid and the acids and potassium salts of several members of the naphthyl series of root growth stimulating chemicals. The higher members of the series compared favourably with naphthylacetic acid in respect to most of the responses considered, though there were indications that naphthylacetic acid or its salt had a greater effect on the number of roots per rooted cutting. Naphthylacetic and 1-γ-naphthylbutyric members of the series were equally effective as acids or as salts; however, a mixture of the isomeric 1- and 2-γ-naphthylbutyric acids was more active than the corresponding mixture of salts. Conversely, potassium naphthylhexoate appeared to have greater activity than the acid. The results suggest that pure naphthylbutyric acid, the isomeric mixture of acids, and potassium naphthylhexoate are virtually as effective as the recognized plant growth stimulating chemicals, indolyl- and naphthylacetic acids. Noteworthy was the beneficial effect of treatment with talc alone, particularly as regards promotion of new growth and rooting of dormant cuttings. B. J. R.

Wood Anatomy and Pollen Morphology in the Anacardiaceæ.—C. Heimsch Jr. ("Wood Anatomy and Pollen Morphology of *Rhus* and Allied Genera," *J. Ann. Arb.*, 1940, **21**, 279–91, 3 pls.). Study of the secondary xylem and the pollen grains of the *Rhus* complex supports Barkley's grouping under six genera: *Rhus*, *Cotinus*, *Toxicodendron*, *Metopium*, *Malosma*, and *Actinocheita*.

B. J. R.

Wood Structure of the Bignoniaceæ.—S. J. Record and R. W. Hess ("American Timbers of the Family Bignoniaceæ," *Trop. Woods*, 1940, **63**, 9–38). This paper describes the general properties and structure of the woods of the sixteen genera of tree size in the New World. There is a key based on the microscopic structure of the woods. B. J. R.

CRYPTOGAMIA.

Pteridophyta.

Apogamy in Pteris.—Alphonse L. Heun ("The Cytology of Apogamy in *Pteris cretica* Linn. var. *albo-lineata* Hort.," *Bull. Torrey Bot. Club*, 1939, **66**, 549–62, 2 pls.). The phenomenon of apogamy in a fern was clearly described for the first time in 1874 by Farlow; the fern investigated being *Pteris cretica* var. *albo-lineata*. The present author has now studied the cytology of the same plant in this process. The antheridia of the gametophyte develop normally, but all the archegonia abort. The nuclei of the gametophyte cells contain 32 chromosomes. There is no fusion of gametophyte cells and their nuclei before the apogamous embryo begins its development. The sporophyte is always of apogamous origin and retains the reduced number of chromosomes. In each sporangium eight spore mother-cells are produced; the spores are formed without any previous meiotic divisions; the mother-cells are haploid, containing 32 chromosomes; the mother-cells appear to pass through synapsis, but there is no pairing of chromosomes; two meiotic

divisions result in the formation of 32 haploid spores. No fusions of sporogenous cells were observed. Abortion of sporogenous tissue may occur at any stage of development—between the archesporial cell and spore formation; such abortion results in the disintegration of the nuclear and cytoplasmic material.

A. G.

Angiopteris.—ARTHUR W. HAUPT ("Sex Organs of *Angiopteris evecta*," *Bull. Torrey Bot. Club*, 1940, **67**, 125–9, 16 figs.). A study of gametophytes of *Angiopteris* collected in Samoa in 1912, giving additional details about the development of the archegonium and antheridium. The development of the antheridium is similar to that of the other Marattiaceæ. The archegonium develops without the formation of a basal cell. The formation of the central canal cell and egg precedes the division of the nucleus of the neck canal cell. A single binucleate neck canal cell is present (rarely two cells). Several archegonia may ripen at the same time, but fertilization results in the degeneration of all of the archegonia except the one fertilized.

A. G.

Polypodium.—HERMANN WOLF ("Studien an *Polypodium vulgare* L.", *Hedwigia*, 1940, **79**, 1–64, 33 figs.). A detailed discussion of the abnormalities sometimes found in the common fern *Polypodium vulgare*, arranged under the headings: dichotomy of the sporophyll; pinnation of the sporophyll; nervation of the sporophyll; position and shape of the sorus; the rhizome. A bibliography of eighty-four publications is appended.

A. G.

Azolla Sporangium.—ROBERT E. DUNCAN ("The Cytology of Sporangium Development in *Azolla filiculoides*," *Bull. Torrey Bot. Club*, 1940, **67**, 391–412, 30 figs.). The results of this investigation indicate that the sporocarp initial is foliar in origin and replaces the lower lobe, while the hood is an outgrowth from the upper lobe. The "sex" of the sporocarp depends upon whether or not the terminal first-formed macrosporangium persists. The large number of nuclei in the tapetal plasmodium is accounted for by two waves of mitotic divisions: the first at the time of synesis in the spore mother-cell nuclei; the second after the tapetal plasmodium is formed. The formation of massulæ (as well as of the swimming organs and epispore of the macrospore) involves a disintegration of plasmodial nuclei; and a subsequent decadence of the plasmodial cytoplasm leads to vacuolation and the formation of the platelets of the massulæ. Glochidia are formed from nucleated cytoplasm on the massular surfaces. As to somatic divisions, about forty short chromosomes can be distinguished; prochromosomes are present in the nuclei in regions of active growth. Meiosis seems to be typical; from eighteen to twenty pairs of chromosomes can be seen.

A. G.

Bryophyta.

Pellia.—HANS BLEHER ("Über Membranverdickungen im Füllgewebe bei *Pellia epiphylla*," *Hedwigia*, 1940, **79**, 65–71, 11 figs.). Description and figures of the thickened bands which are found in the inner parenchymatous tissue of the thallus of *Pellia epiphylla*. The straight, spiral, and annular elements of the bands unite to form a strengthened reticulum which saves the thallus from collapse in times of drought. They also occur in *P. Neesiana* but not in *P. Fabbroniana*.

A. G.

Plagiochila.—TH. HERZOG ("Beiträge zur Kenntnis der Gattung *Plagiochila*. II. Palæotropische Arten," *Hedwigia*, 1938, **78**, 222–44, 15 figs.). Descriptions and figures of fourteen new species and some new varieties of *Plagiochila*, mostly from

the East Indies. The type specimen of *P. semidecurrens* Legm. & Lind. (1844) is also the subject of some critical notes, in which the distinctive characters of the plant are emphasized. A. G.

Notes on Hepatics.—KARL MÜLLER ("Beiträge zur Systematik der Lebermoose," *Hedwigia*, 1940, **79**, 72–80, 1 fig.). Five plants are discussed in this paper: *Sauteria quadrata* Sauter; *Clevea Spathysii* (Lindenberg) comb. nov.; *Clevea hyalina* var. *Kernii*, *Targionia Lorbeeriana* sp.n., *Calypogeia Mülleriana*. *Sauteria quadrata* published by Sauter in 1871 ("Flora die Herzogtums Salzburg," iv., p. 27) and previously described in 1858 ("Flora," xli., p. 385) as perhaps a form of *Preissia quadrata*, has hitherto been overlooked by hepaticologists. All the specimens of it in Sauter's herbarium, preserved at Vienna, have been carefully examined by the author, who is able to show that the plant described by Sauter is identical with *Sauteria grandis* Lindberg (1876), which is the type of the genus *Peltolepis* Lindberg (1882). On the ground of priority the new combination *Peltolepis quadrata* (Sauter) is proposed. A. G.

Thallophyta.

Algæ.

New Myxophyceæ.—GEORGE J. HOLLENBERG ("Some New Myxophyceæ from Southern California," *Bull. Torrey Bot. Club*, 1939, **66**, 489–94, 8 figs.). Descriptions and figures of the following new species: *Myxohyella seriata*, *Entophysalis marginalis*, *Xenococcus pulcher*, *Microcystis splendens* and *M. ovalis*. A. G.

Fossil Desmids.—EDWIN MESSIKOMMER ("Beitrag zur Kenntnis der fossilen und subfossilen Desmidiaceen," *Hedwigia*, 1938, **78**, 107–201, 1 fig., 9 pls.). The results of the author's investigations are presented under the following headings: (1) Fossils and fossilization; (2) Fossils of algæ other than desmids [e.g. coccoliths, diatoms, corallines]; (3) Fossil and subfossil desmids [subfossil means postglacial]; (4) Technical methods employed in the research; (5) List of recorded fossil and subfossil desmids, with notes as to locality, geological stratum, discoverer, date; (6) Remarks on the algæ figured [the 9 plates contain 114 figures]; (7) Summary and conclusion. Appended is a bibliography of 205 publications. A. G.

Tetraëdron.—CHARLES B. REIF ("A New Species of *Tetraëdron* from Minnesota," *Bull. Torrey Bot. Club*, 1939, **66**, 615–16, 10 figs.). Description and figures of *Tetraëdron grande*, a Chlorococcal alga found in four eutrophic lakes of west-central Minnesota. It is closely related to *T. quadricuspidatum*. A. G.

Codium.—O. C. SCHMIDT ("Über *Codium amphibium* Moore," *Hedwigia*, 1939, **78**, 268–72, 3 figs.). A detailed description and figures of the structure of this rare little species of *Codium* are given. It has been recorded from two places on the west coast of Ireland, from the Isle of Man, and from Cornwall; its distribution is now extended to Calvados, in Normandy, where it has been under observation for some years. The fact that it is an annual plant and very small may explain its reputed rarity. A. G.

Swedish Algæ.—O. BORGE ("Beiträge zur Algenflora von Schweden," *Arkiv. för Botanik*, 1939, **29A**, No. 16, 1–26, 14 figs.). A posthumous paper, the first part of which comprises lists of freshwater algæ collected in various parts of Sweden, partly by the author, partly by other botanists. The second half is a systematic account of the more interesting forms which either are new to the Swedish flora or are of rare occurrence, with short descriptive and critical notes, and remarks on distribution. A. G.

Indian Algæ.—M. S. RANDHAWA ("Observations on some new and interesting Algæ from Northern India," *Hedwigia*, 1939, **78**, 273–83, 6 figs.). The following freshwater algæ from the district of Fyzabad are figured and described or discussed: *Microspora indica* sp.n., *Cylindrocapsa oedogonioides* Randhawa, *C. scytonemoides* sp.n., *Botrydium tuberosum* Iyengar, *B. divisum* Iyengar, *Oedogonium terrestris* sp.n. The discovery of a terrestrial species of *Oedogonium* is a surprise; it is a typically monsoon alga, appearing in early rains and disappearing at the close of the rains in October; its habitat is the sandy soil of lawns and compounds.

A. G.

Lichens.

Lichens of Formosa.—M. SATÔ ("Enumeratio Lichenum Ins. Formosæ," *Journ. Jap. Bot.*, 1938, **14**, 463–9 and 783–91, 8 text-figs.). The fourth and fifth continuations of the author's taxonomic treatment of the lichens of Formosa. One of them deals with the *Usnea*-species; these are seven in number, and the structure of the thallus in transverse section is found to be a valuable characteristic for their identification. In the fifth continuation four species of *Nephromopsis*, eight of *Cetraria*, and four of *Anzia* are enumerated. I. M. L.

Fungi.

Sapromyces.—H. BISHOP ("A Study of Sexuality in *Sapromyces Reinschii*," *Mycologia*, 1940, **32**, 505–30, 6 figs.). *Sapromyces Reinschii* from different sources was isolated and grown in pure culture. Favourable conditions for the development of mycelia, zoosporangia, and sexual organs were determined and the sexuality of the strains was studied in detail. Of seventeen isolates from single zoospores studied intensively, four were strongly male, one weakly male, five neuter, one weakly female and six strongly female. The female strains consistently showed latent maleness and could develop antheridial branches and even accomplish self-fertilization. Latent femaleness was not observed in the male strains. Six possible types of sexuality are postulated for this fungus: (1) pure male, (2) male with latent femaleness, (3) neutral, strongly sexed, (4) neutral, weakly sexed, (5) female with latent maleness, and (6) pure female. Of these (1), (4) and (5) were represented in the strains studied.

F. L. S.

Phytophthora.—G. W. GIBSON and P. H. GREGORY ("A *Phytophthora* Blight of Bulbous *Iris*," *Tr. Brit. Myc. Soc.*, 1940, **24**, 251–4, 5 text-figs.). An account of a leaf blight of several varieties of Dutch *Iris* which has occurred in the Scilly Isles since 1928. The *Phytophthora* causing the disease appears to be related to *P. Cyperi-rotundati* Sawada. Control measures are suggested. F. L. S.

British Pyrenomycetes.—G. R. BISBY and E. W. MASON ("List of Pyrenomycetes Recorded for Britain," *Tr. Brit. Myc. Soc.*, 1940, **24**, 127–244). This list of British Pyrenomycetes is prefaced by a short introduction. The list is arranged in alphabetical order of genera in each family, with references to literature giving new information or interesting taxonomic or biological points. An index to the genera and species completes the paper. F. L. S.

Gelasinospora.—E. S. DOWDING and A. H. R. BULLER ("Nuclear Migration in *Gelasinospora*," *Mycologia*, 1940, **32**, 471–89, 6 figs.). Migration of nuclei from the mycelium of one sex to the other was observed in *Gelasinospora tetrasperma*. Light influenced this migration, the nuclei moving from a darkened mycelium of one sex toward the illuminated part of a mycelium of the opposite sex. F. L. S.

Caryospora.—W. F. Jeffers ("Studies on *Caryospora putaminum*," *Mycologia*, 1940, **32**, 550–67, 2 figs.). *Caryospora putaminum* which occurs chiefly on old peach stones but also on acorns, hickory nuts, etc., was studied from the cytological, cultural, and anatomical standpoints. During these studies another member of this genus was found which differing from hitherto described species is named *C. minima* sp. nov.
F. L. S.

Rusts.—Malcolm Wilson ("The British Species of *Puccinia* included under '*P. Syngenesiarum*,' with Notes upon the British Rust Fungi occurring on Thistles," *Tr. Brit. Myc. Soc.*, 1940, **24**, 244–51). Eight specimens of *Puccinia Syngenesiarum*, the identity of which was very doubtful, were examined and fifteen proved to be *P. Le Monnieriana* Maire on *Cnicus palustris*. Notes on the nomenclature and distribution are given of *P. Cnici-oleracei* Pers., *P. Cardui-pycnocephali* Sydow, *P. Cnici* Mart., *P. Cirsii* Lasch, *P. Cirsii-palustris* (Desm.) comb. nov. and *P. suaveolens* (Pers), Rosts.
F. L. S.

Boletus Development.—R. P. Elrod and W. H. Snell ("Development of the Carpophores of Certain Boletaceæ," *Mycologia*, 1940, **32**, 493–505, 2 figs.). Ontogenetic studies are described of five species of Boletaceæ; three exannulati species of the Viscipelles group and two of Boletinus.
F. L. S.

Basidiomycete Evolution.—D. H. Linder ("Evolution of the Basidiomycetes and its Relation to the Terminology of the Basidium," *Mycologia*, 1940, **32**, 419–48, 6 figs.). The evolution that has led from the Uredinales to the higher Basidiomycetes has resulted from changes in life-cycles, in time and position of nuclear divisions and in mode of living. Many forms to-day have retained several primitive characters while possessing others more advanced. The haploid phase has gradually become less important and the dikaryon has become dominant, while spermatization has given place to plasmogamy. The basidium has become simplified by loss of septation and in the higher forms has assumed the functions of the probasidium, so that it becomes the locus of both caryogamy and meiosis. The author advocates the older and simpler terminology instead of the numerous descriptive terms, probasidium, hypobasidium, metabasidium, promycelium, etc., now in vogue.
F. L. S.

New Genus.—E. S. Luttrell ("An Undescribed Fungus on Japanese Cherry," *Mycologia*, 1940, **32**, 530–7, 10 figs.). A Melanconiaceous fungus distinguished by its pleurogenously formed conidia on multicellular coniodiophores was found infecting twigs of Japanese Cherry, *Prunus serrulatus* in North Carolina. The fungus has been placed in a new genus *Catenophora* and named *C. Pruni*.
F. L. S.

Helminthosporium.—K. Sampson and J. H. Western ("Two diseases of Grasses caused by species of *Helminthosporium* not previously recorded in Britain," *Tr. Br. Myc. Soc.*, 1940, **24**, 255–64, 2 text-figs.). *Helminthosporium siccans* Drechsler on *Lolium perenne*, *L. multiflorum* and *Festuca pratensis* and *H. vagans* Drechsler on *Poa pratensis* are recorded for the first time for Britain. Single spore cultures of both fungi were studied.
F. L. S.

New Hyphomycetes.—C. Drechsler ("Three New Hyphomycetes Preying on Free-Living Terricolous Nematodes," *Mycologia*, 1940, **32**, 448–71, 3 figs.). Three Hyphomycetes, *Dactyella doedycoides*, *Dactylaria haptospora* and *Tridentaria implicans*, were found in agar cultures started from discoloured vegetable remains. These organisms subsisted by the capture of free-living terricolous eel-worms. They are described as new to science.
F. L. S.

Entomogenous Fungus.—V. K. CHARLES ("The Entomogenous Fungus on Spider Mites on Water Hyacinth," *Mycologia*, 1940, **32**, 537–41). A fungus, named *Rhinotrichum depauperatum* sp. nov. was found on spider mites, *Paratetranychus yothersi* on water hyacinth in Maitland, Florida. In the natural condition the mites were enmeshed in a web of white mycelium. F. L. S.

Bulb Disease.—C. S. MACFARLANE ("A Rot of *Scilla* Bulbs caused by *Penicillium cyclopium* Westling," *Tr. and Proc. Bot. Soc. Edinburgh*, 1939, **32**, 542–8). Bulbs of *Scilla campanulata* var. *albida* from Holland were attacked by a species of *Penicillium*, the cultural characters of which indicated that it was *P. cyclopium* Westling. Infection experiments showed that injury and an external source of moisture were necessary before infection occurred. F. L. S.

Florida Fungi.—W. H. MURRILL ("Additions to Florida Fungi—V," *Bull. Torrey Bot. Cl.*, 1940, **67**, 275–83). An account of fourteen fungi new to science. The species are distributed among the genera *Galera*, *Flammula*, *Tricholoma* and *Hydnum*. F. L. S.

BOOK REVIEWS.

Electron Optics: Theoretical and Practical.—By L. M. Myers. 1939. xviii+618 pp., 380 illustrations, including 68 plates. Published by Chapman & Hall, Ltd., London, W.C.2. Price 42*s*. net.

It is probable that the coming years will see an increasing interest in this subject, and the Royal Microscopical Society, if it takes advantage of its opportunities, may take a prominent part in its development. To the ordinary microscopist, perhaps, this book is rather in advance of his immediate wants, but there is no question that it provides all that is needed for a good knowledge of the subject. Electron microscopy is founded on the principles of pure geometric optics; in its essentials, therefore, it is similar to ordinary microscopy The first chapter deals with wave motion and the analogies between light and electrons, an interesting introduction to the whole subject. Then follow five chapters on various technical problems, in which the microscopist is not primarily interested but which may be studied with interest and advantage. Chapter VII deals especially with the electron microscope. Its similarity in purpose is indicated and the changes in design to enable electrons to be used instead of ordinary light are indicated. Perhaps the necessity of mounting the object in a vacuum chamber is one of the greatest difficulties, as not only must the vacuum be maintained but it must not alter. In an experimental instrument constructed in this country this maintenance of a constant vacuum has not been the least of the difficulties encountered. The lens system, by means of which the electron wave-front is caused to expand as in any ordinary optical system, consists of either a magnetic or electrostatic lens. The magnetic system can achieve a higher numerical aperture, although the difficulty of eliminating aberrations is considerable, whereas the electrostatic system, with its smaller attainable aperture, is easier to construct. There is here a certain analogy with the development of the ordinary microscope; large N.A. has been attained, but always with greater difficulty. Magnification is secured through two stages, again suggesting an ordinary optical microscope, with its objective for initial magnification and an ocular to amplify the primary image. In the electron microscope this enlargement of the image is also secured in two stages. A description is given of the Metro-Vick electron microscope constructed for the Department of Physics at South Kensington and described in detail in the *Journal of Scientific Instruments* and in our own Journal by Dr. L. C. Martin. Apparently the latest form of electron microscope described in this book is called an "ultra-microscope," a term that is used in quite a different connection in microscopy and that may easily lead to confusion. A magnification as high as 100,000 diameters is regarded as a possibility, but there is no suggestion in the results shown that there is yet comparable resolution. The whole subject is vastly interesting, abounding in problems new to the microscopist of the older school. Its full possibilities will almost certainly be attained only by team work, with each worker competent to deal with a highly specialized branch of the physical and biological questions involved. The book is a complete survey of the subject, is well produced, and will almost certainly be regarded as a welcome addition to the limited literature at present available in the English language.

J. E. B.

The Microscope.—By R. M. ALLEN. 1940. viii+286 pp., 82 text-figs., 17 plates. Published by Chapman & Hall, Ltd., London, W.C.2. Price 15*s*. net.

A new book on the microscope is badly needed but, to appeal to English readers, it must be one with a different outlook from that now under review. In the preface the author refers to this limitation, and attributes it to English manufacturers who do not make their products known to American users, although he admits that English instruments are often very "appealing to discriminating microscopists". He does not, however, say how very difficult it is for *unsubsidized* makers to distribute their products in countries where prohibitive import duties operate so strongly against them. To some readers the advantage will lie in providing an account of the choice afforded to American workers who wish to rely largely on home products. In the general description of microscope design reference is made to the specification of the Royal Microscopical Society standard screw thread for objectives, which has been universally adopted in all countries. This is correctly stated as a footnote on page 41, but on the next page a reference is made to the "Royal Society" thread, which is misleading, as the latter society has taken no part in microscope design, nor, indeed, is it likely to do so. This error recurs at intervals where reference to the Royal Microscopical Society is intended. Recent changes in design are described and types are illustrated from makers' catalogues. The recent tendency to reverse the disposition of parts does not receive whole-hearted approval, which is evidence that the writer is an experienced worker. He rightly refers to the difficulty of changing over to this new type, when, as often occurs, both orthodox and reversed type microscopes are in use in the same laboratory, also stressing the greater adaptability of the older design. The term "critical illumination" is used, and is associated in some way with Abbe, but with little suggestion that the author really appreciates the significance of the term. The chapter on "Getting the most out of the microscope" is very useful, there are many hints and detailed instructions that are often superficially treated by writers on the subject. The section on preparation of material is good but does not pretend to be exhaustive. The book is well printed on very good paper.

J. E. B.

PROCEEDINGS OF THE SOCIETY.

For the information of the Fellows the Council authorizes the publication of the following condensed report of the Society's proceedings since that published in the last issue of the *Journal*.

New Fellows.—The following have been elected Ordinary Fellows of the Society :—

Frederick James Burke.	Liverpool.
George William Davis.	Birmingham.
William Joseph Friskney.	Newton Abbott.
John J. Grover.	Rotherham.
Henry Chapman Pincher, B.Sc.	Liverpool.
Edward V. F. Pittock.	Croxley Green.
Arthur Davison Simms.	Potters Bar.
Edward Wilfred Taylor.	York.

Deaths.—In addition to those recorded in the Annual Report of the Council (see p. 72) the death of the following Fellow is reported :—

G. H. Nall. Elected 1914.

Donations.—The following donations have been received and duly acknowledged on behalf of the Fellows :—

McGraw-Hill Publishing Co., Ltd.

"Plant Microtechnique." By D. A. Johansen.

Medical Research Council.

"Chemical Composition of Foods." By R. A. McCance and E. M. Widdowson.

Chapman & Hall, Ltd.—

"Practical Microscopical Metallography." By R. H. Greaves and H. Wrighton. Third Edition.
"Laboratory Manual for General Bacteriology." By G. L. Peltier, C. E. Georgi, and L. F. Lindgren. Second Edition.
"Fluorescence Analysis in Ultra-Violet Light." By J. A. Radley and Julius Grant. Third Edition.
"The Microscope." By R. M. Allen.
"Insect Pests in Stored Products." By H. Hayhurst.

C. T. Owen, F.R.M.S.—

"An Essay towards a Natural History of the Corallines." By John Ellis, F.R.S. 1755.

4 bone slides (one circular) with mica cover slips.

2 small hand microscopes.

A. A. C. Eliot Merlin, F.R.M.S.—

Collection of unpublished MS. on Microscopic Optics by the late E. M. Nelson.

S. C. Akehurst, F.R.M.S.—

"A Treatise on Optics." By David Brewster. 1831.

W. G. Paterson.—

A pocket microscope by Francis Watkins, *c.* 1750, with accessories in shagreen case.

Dingley P. Fuge, F.R.M.S.—

20 slides of Diatoms.

Evan G. MacLeod, F.R.M.S.—

(Three guineas) £3 3*s.* 0*d.*

Grants in aid of Publications.—

(Sixty pounds) £60 0*s.* 0*d.*

Papers.—The following papers received have been considered and approved by Council for publication in the Society's *Journal* :—

Ernest A. Gray, M.R.C.V.S., F.R.M.S.—

"Some Ecological Observations upon the Infusoria."

Walter Koch, M.D., Ph.D.—

"Increasing the Depth of Focus for Photomicrography by Incident Light."

Prof. H. Graham Cannon, F.R.S., F.R.M.S.—

"A Note on Fine Needles for Dissection."

J. M. Watson, A.R.C.S.—

"Studies on the Morphology and Bionomics of a little known Holotrichous Ciliate—*Balantiophorus minutus*, Schew. 1. Structure and Relationships."

P. B. Medawar, M.A.—

"The Rate of Penetration of Fixatives."

Miss F. M. L. Sheffield, D.Sc.—

"The Cytoplasmic and Nuclear Inclusions associated with Severe Etch Virus."

Adrianus Pijper, M.D., D.Sc., F.R.M.S.—

"The Microscope in Biology."

101st ANNUAL REPORT.

REPORT OF THE COUNCIL FOR THE YEAR 1940.

The Council orders the publication of the following Annual Report on the affairs and work of the Society during the past year for the attention of the Fellows. For reasons of economy and other circumstances due to the War the report has been curtailed and is shorter than would otherwise have been contemplated.

PRESIDENT, OFFICERS AND MEMBERS OF COUNCIL.

In accordance with Bye-Laws 41–42, and the resolution passed at the last Annual Meeting of the Society, the last elected President, Officers, and Members of Council continue to serve in office with the exception of those changes noted below.

The present personnel of the Council is as follows :—

PRESIDENT,

J. E. Barnard, F.R.S.

VICE-PRESIDENTS,

Dr. R. S. Clay.
M. T. Denne, *O.B.E.*
Dr. G. M. Findlay, *C.B.E.*
D. J. Scourfield, *I.S.O.*

HON TREASURER,

C. F. Hill.

HON. SECRETARIES,

Dr. G. M. Findlay, *C.B.E.*
(One office vacant.)

ORDINARY MEMBERS OF COUNCIL,

C. Beck, *C.B.E.*
R. J. Bracey.
Dr. H. M. Carleton.
Prof. R. R. Gates, F.R.S.
N. Ingram Hendey.
Dr. R. J. Ludford.
Dr. J. E. McCartney.
Dr. J. A. Murray, F.R.S.
J. H. Pledge.
T. E. Wallis.
H. Wrighton.
S. R. Wycherley.

The changes effected during the year are as follows:—Mr. J. Smiles has relinquished his office as Joint Honorary Secretary, and Dr. G. M. Findlay has been appointed in his place.

The vacant Honorary Secretaryship caused by the recent death of the late Prof. R. T. Hewlett has not yet been filled.

The following continue to serve in their respective offices :—*Hon. Editor*, Dr. G. M. Findlay, *C.B.E.*; *Hon. Curator of Instruments*, Mr. M. T. Denne, *O.B.E.*; *Hon. Curator of Slides*, Mr. N. Ingram Hendey; *Secretary and Librarian*, Dr. C. Tierney.

FELLOWS.

The Council deplores the loss the Society has sustained by the death of one of its Honorary Secretaries, Prof. R. T. Hewlett, and of the following Fellows :—

Maurice Blood.		Elected 1903.
L. G. Gilpin-Brown.		,, 1921.
Sir Robert Hadfield.	(Hon. Fellow.)	,, 1933.
F. H. Lewis.		,, 1927.
A. J. Murphy.		,, 1900.
Moritz von Rohr.		,, 1930.

Fourteen Fellows have resigned, and thirteen have been removed from the Roll of Fellowship under By-Law 31.

One Fellow has been reinstated and four new Ordinary Fellows have been elected.

MEETINGS.

Owing to the prevailing hazardous conditions which render the postponement of meetings advisable, and to the fact that so many Fellows are fully engaged on work in connection with the War, adequate attendance was not to be expected and only one meeting has been convened.

In view of these considerations, and of the Society's obligations under its Charter and By-Laws, an Appeal to the Privy Council on behalf of the Society was lodged in November last, and subsequently granted, the terms of which are fully stated in the following direction :—

> . . . the Lord President of the Council, under the powers conferred upon him by the Chartered and Other Bodies (Temporary Provisions) Act, 1939, and the Order in Council made thereunder, has been pleased to direct that for the period during which the above-mentioned Act remains in force, and notwithstanding anything contained in the Charter or Bye-laws of the Royal Microscopical Society, the following provisions shall have effect :—
>
> 1. The Council shall have power to suspend the Annual Meeting of the Society.
> 2. Where any action is required by the Bye-laws to be taken at or in connection with the Annual or other ordinary meeting of the Society, the Council may take the action or direct that it be taken at such time as they may determine, or be deferred for any period during the continuance of this direction : Provided that this direction shall not enable the Council to amend the Bye-laws of the Society.
> 3. The Council shall have power to modify, in their discretion, the conditions governing the retirement of officers and members of the Council.

The Council regrets that prevailing conditions have rendered it necessary to act in accordance with the foregoing provisions and to suspend the Annual Meeting.

Nevertheless it records with satisfaction that the Executive, Editorial, and other activities of the Society continue to be carried on without intermission. The Society's offices and Library have remained open throughout the year and a notable feature of the period has been the increased number of volumes borrowed from the Library, and the additional amount of correspondence and inquiries dealt with in the Society's office for information on problems connected with technical and applied microscopy in scientific and in industrial processes.

LIBRARY, INSTRUMENT, AND SLIDE COLLECTIONS.

Fourteen new volumes have been presented for addition to the Library, and twenty species slides of Diatoms have been added to the cabinet.

Three small early microscopes, one modern microscope, and Powell & Lealand's first $\frac{1}{40}$th apochromatic objective have been added to the instrument collection.

The thanks of the Fellows have been duly conveyed to the donors.

JOURNAL.

The publication of the Society's *Journal* has continued throughout the year. The conduct of this important work involves no inconsiderable amount of time and labour on the part of those responsible for its efficient execution, whose valued services to the Society the Council gratefully acknowledges.

In addition to the scientific papers published it is gratifying to report that, despite the difficulties, the publication of the series of abstracts from British and foreign scientific journals has been maintained.

The thanks of the Fellows are especially due to the Honorary Editor, Dr. G. M. Findlay, and to the Abstractors, for their support under exceptionally arduous conditions.

Owing to the necessary conservation of paper supplies, and to the considerably increased costs of printing and publishing, it is regretted that several of the scientific papers contributed in honour of the Society's Centenary, including the President's Address, have unavoidably been held over. These will be included in the next volume.

The Council also regrets to note a material loss of revenue normally received in respect of the *Journal* from continental academies and scientific bodies at present under enemy domination.

LECTURES TO HIS MAJESTY'S FORCES.

Under the auspices of the Universities, Regional Committees have been set up during the year throughout the country for providing educational lectures among His Majesty's Forces. Lists of names of those Fellows of the Society signifying their willingness to give occasional lectures or talks under this scheme at the various camps and depots have been forwarded to the respective committees. More offers would be warmly welcomed, and those willing to assist are invited to send in their names to the Secretary.

Concerning the War and its effects upon the Society, these cannot yet be assessed. It is sufficient to say that the Society's activities continue undeterred notwithstanding the very real difficulties and menacing conditions under which these have to be conducted at the present time.

The Council is assured of the importance of this work and of the universal value of the Society's contribution to the study and advancement of microscopical science. In their determination to carry on and maintain in this country the work for which the Society was founded more than one hundred years ago, and to which with unremitting devotion and achievement the Fellows have ever since applied themselves, the Council relies with confidence upon their continued co-operation and support.

JOURNAL

OF THE

ROYAL MICROSCOPICAL SOCIETY.

SEPTEMBER & DECEMBER, 1941.

TRANSACTIONS OF THE SOCIETY.

V.—A FLUID FOR SOFTENING TISSUES EMBEDDED IN PARAFFIN WAX. 535.826.3

By John R. Baker, M.A., D.Sc.

(From the Department of Zoology and Comparative Anatomy, Oxford.)

When material embedded in paraffin wax is so brittle or crumbly that good sections cannot be obtained, it is often helpful to soak the block in water. Some workers have thought that the water acts by cooling the paraffin, but the method does not work unless the tissue itself is directly exposed to the water. It is well known that water can penetrate into tissues embedded in paraffin wax. When paraffin sections of material fixed in a fluid containing picric acid are being flattened on warm water, part of the picric acid often leaves the tissue and slightly colours the water. Conversely, one can stain sections still embedded in paraffin wax with dyes dissolved in water (McFarland, 1922; Walsem, 1930).

Experiments were made to find whether a fluid could be designed which would be preferable to water for the purpose of softening tissues embedded in paraffin wax. For this purpose mammalian liver was chiefly used, on account of its tendency to be a difficult tissue to section. Mammalian pancreas and kidney and other tissues were also tried. Picro-formol-alcohol (Rossman, 1940), excellent for the preservation of glycogen, was generally used as a fixative, because it tends to make tissues brittle or crumbly unless special precautions are taken. Formol-saline and Zenker's fluid without acetic acid were among the other fixatives used in this investigation.

The experimental method adopted was to make a number of strictly comparable paraffin blocks of the same tissue, expose the tissue in each block by a knife-cut, and soak the blocks in different fluids for the same

length of time (generally about 24 hours). Each block was then wiped dry and sectioned at the same thickness as the others (generally 10μ), careful note being made of the cutting qualities at the start, of the cutting qualities when 100 sections had been made, and of the number of sections that had been cut when holes first appeared in the sections and when the sections began to roll up badly.

The best fluid in these respects among those under test in any given experiment gave an indication as to what further experiments should be made to improve the fluid. Various mixtures of the following substances were tried :—distilled water, alcohol, glycerine, diacetine, acetone, acetic acid, lactic acid, triethanolamine, sorbitol, iodine (in potassium iodide solution), ethyl resorcinol, propylresorcinol, hexylresorcinol, saponine, sodium taurocholeate, and carbitol.

From the experiments made it became apparent that water and glycerine are the best softeners. The latter is much the more effective, but also the slower in penetration, and it was necessary to make a compromise by including both substances. The only other substance among those listed above which was proved to be definitely helpful was alcohol, which increases the rate of penetration of mixtures of glycerine and water and, at appropriate concentrations, has the three further advantages of avoiding maceration on prolonged soaking, of preventing the removal of glycogen from the tissues, and of fixing any proteins that may have been set free from combination with lipides by the action of the de-alcoholizing fluid (benzene or xylene). As regards the last-named advantage, Carleton and Leach (1937) have suggested a method for using diacetine for the same purpose.

Experiments showed that the best proportions in which to mix water, alcohol, and glycerine were the following :

Alcohol, 60 p.c. aqueous	90 c.c.
Glycerine	10 c.c.

If 60 p.c. alcohol is not available, one may take 56 c.c. of 96 p.c. alcohol and make up to 90 c.c. with distilled water.

The fluid penetrates well and gives limp, easily flattened sections even from brittle glycogen-liver. The alcohol is at sufficient concentration to avoid maceration and to prevent the extraction of glycogen and water-soluble proteins and to facilitate penetration, but not concentrated enough to interfere seriously with the softening effect. If the amount of glycerine be increased, the tissues will be still softer, but penetration will be slower. If it is desired, the proportions may be varied within fairly wide limits.

It is surprising that the substances tried on account of their surface-action were not found to be demonstrably helpful. Good results were often obtained when 0·2 p.c. of propylresorcinol was added to the fluid, but this did not happen regularly enough to warrant the statement that it is definitely helpful. Propylresorcinol has the disadvantage of rendering the fluid unstable, so that slight discoloration occurs in a few weeks.

It must be admitted that when tissues are tough, owing to abundance of connective tissue or muscle, soaking in the fluid is not helpful. No amount of treatment with any fluid known to the writer will render it easy to make paraffin sections of, e.g., the vagina of a bitch after formalin fixation. The fluid described in this paper is especially applicable to epithelial tissues and those in which there is brittleness due to the presence of large amounts of blood. Above all, it is successful with pieces of liver prepared for the demonstration of glycogen.

The most convenient way of using the fluid is as follows. The paraffin block is attached to the microtome chuck in the usual way, and sections are cut until the tissue is well exposed. If the material is found to be brittle or crumbly, or if holes appear in the sections or the sections tend to roll up on the knife or fail to flatten easily on the hot plate, immersion in the fluid should be tried. The chuck carrying the paraffin block is removed from the microtome, and the block, still attached to the chuck, is placed in the fluid in such a way that the exposed surface of the tissue is bathed in the fluid. Soaking should proceed for a few hours or longer, according to the number of sections that it is desired to cut. The rate of penetration varies widely according to the nature of the tissue and the fixative used, but an average would be about 3 mm. in 24 hours, so that four hours will generally suffice if only a few dozen 10μ sections are required. As a general rule, it is convenient to leave the block in the fluid overnight. The block is wiped dry and the chuck replaced on the microtome. The first sections may be discarded if slight swelling of the tissue has occurred.

An alternative method is to soak blocks in the softening fluid before attaching them to the microtome chuck. Whichever method is adopted, the full exposure of a surface of the tissue by a razor cut is necessary.

The fluid has no damaging effect on delicate cytological structure. Spinal ganglia of the cavy prepared by Weigl's method and the intestinal epithelium of the newt prepared by Nassonov's were embedded in paraffin wax and exposed to the fluid overnight in the usual way. Both blocks provided good preparations of the Golgi element. Liver of the white mouse was fixed in Helly's fluid, postchromed, embedded in paraffin and exposed overnight to the fluid. The sections were stained by Volkonsky's method and showed the mitochondria well. The fluid leaves the tissues in a convenient state for the preparation of thin sections.

The physical properties of the fluid make it useful to the histologist in quite another way. It is a very convenient medium for smearing over glass vessels in which objects are about to be embedded in paraffin wax. The glass is lightly smeared with a few drops of the fluid, melted paraffin is poured in, the object is arranged in position and the whole immersed in cold water in the usual way. The congealed paraffin will usually float out of the glass vessel unaided; in any event, it will always be easily removed. The fluid is much superior to glycerine for this purpose, on account of its not tending to run into separate drops.

The fluid may also be used in place of alcohol for the storage of tissues when hardening is undesirable.

At the writer's suggestion, the British Drug Houses, Ltd., are putting on the market a fluid similar to, but not identical with, the one suggested in this paper. The name " mollifex " is being used.

Summary.

When tissues embedded in paraffin wax are brittle or crumbly, they may be soaked (e.g., overnight) in the following fluid :

Alcohol, 60 p.c.	90 c.c.
Glycerine	10 c.c.

This penetrates despite the presence of the wax, provided that the latter is cut at one side so as to expose the tissue at the surface. The fluid softens the tissue and makes section-cutting easier. Water has similar effects, but the mixture penetrates faster, makes the tissue softer, avoids maceration, and prevents the extraction of glycogen and water-soluble proteins.

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VI.—THE APPLICATION OF THE MICROSCOPE IN FORENSIC SCIENCE. 343. 9.

By James Davidson, M.B., Ch.B., F.R.C.P., Edinburgh.

(Director of the Metropolitan Police Laboratory.)

(*Centenary Communication, Royal Microscopical Society*, 1839–1939.)

Two Plates.

In past years the scientific investigation of crime was practically limited to those types of crime known as Offences against the Person. These being the most serious crimes involving assault upon an individual resulting possibly in death, would naturally receive more serious consideration, and thus gradually there has evolved an important branch of medicine known as Forensic Medicine which is now a speciality in itself. However, within recent years it has become more and more apparent that all other crimes are worth investigating from the scientific point of view and such investigations have frequently yielded valuable results. Such types of crime constitute the large class known as Offences against Property and are exemplified by house-breaking, safe-breaking, cases of theft and forgery, naturally they constitute a very large proportion of the common everyday types of crime. A wider field is thus covered than that previously included in Forensic Medicine, and this is known as Forensic Science or Scientific Investigation pertaining to the Law Courts.

The microscope as an instrument of investigation in Forensic Science is of primary importance, and it is interesting to review briefly certain of the aspects where its utility is most obvious. In a review such as this it will only be possible to summarize some of the more important of the different types of case to which it is necessary to apply microscopical investigation.

As a rule, very high magnifications are rarely used, the material lending itself chiefly to lower magnifications. Apart from the ordinary hand lens which is in everyday use and is of obvious utility, various forms of instruments are used according to the nature of the investigation. The binocular prism magnifier, for example, is useful for lower power work. It has the advantage of being detachable from a stand and as it can be carried about in the hands in a similar way to field glasses, objects can be viewed stereoscopically at a long working distance. Magnifications from $\times 3$ to $\times 40$ can be obtained with this instrument. It can be taken to the scene of a crime where it can be used in a search for finger prints, blood stains, or other evidence of minute traces. In the laboratory it is frequently

used in this way in a preliminary investigation of clothing or other articles. Mention might be made of its use in shooting cases where it is of importance to make careful observations of the skin in the vicinity of the hole produced by the projectile for the presence of burning or particles of unburned powder whose identification would be of value in estimating the distance from which a firearm has been fired. Free motility of such an instrument is thus of manifold importance where large areas have to be examined. When attached to a stand it can serve the purpose of a dissecting microscope or can be used in the examination of documents in cases of suspected forgery where low-power magnification of hand- or type-writing is required. Another use to which it is put in this laboratory is the comparison of the lines of the spectra in plates taken by the spectrograph—an instrument which is frequently used in the physical examination of material.

The Greenough binocular microscope is of considerable value when somewhat higher magnifications than can be obtained by the low power binocular magnifier are required. It is of special value in cases of forgery where the surface of the paper has to be examined or where very careful examination of writing has to be made.

A microscope which is of considerable importance in forensic science is one of the types of comparison microscopes. As may be fully appreciated, it not infrequently happens that material found in relation to a crime must be compared with other material found in the possession of the suspect or elsewhere; bullets, cartridge-cases, hairs, and fibres are examples. Various types of comparison microscope which range from the simple prismatic attachment between two monocular stands to more complicated types of instrument have been designed. At times it may be necessary to produce a photomicrograph in Court in order that the jury may understand what is being presented to them. One of the difficulties in comparison photomicrography is the illumination system which does not yet appear to have been perfected. It must be of equal and sufficient intensity in either field—a surprisingly difficult thing to obtain.

Another method of enlarging of an object either for purposes of comparison or otherwise is the use of microphotography. Study of the negatives or prints often reveals a considerable amount of information and the prints can readily be shown to an intelligent jury. This method is of value in the comparison of scratch marks made by the tools which are used by the thieving fraternity in obtaining a forced entry into a house or some enclosed area. It can also be used in the comparison of counterfeit coins. Coins produced from the same moulds often show similar characteristic markings. Comparison of printed surfaces such as are seen on postage stamps or paper money are also amongst the many uses to which this method can at times be applied.

It is unnecessary in a paper such as this to enter into a discussion of the various types of illumination used in microscopy, but it has to be realized that in forensic work, a considerable amount of the material which has to be

Fig. 1.

Fig. 2.

(*a*) (*b*)

Fig. 3.

Fig. 4.

[*To face p.* 80.

(a) (b)

Fig. 5.

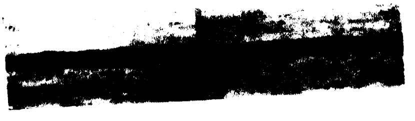
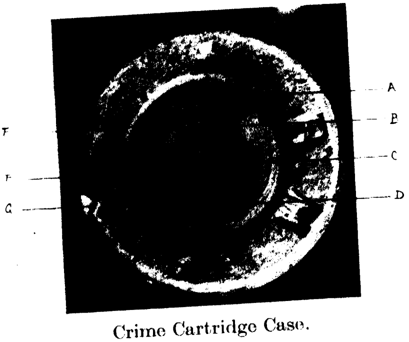
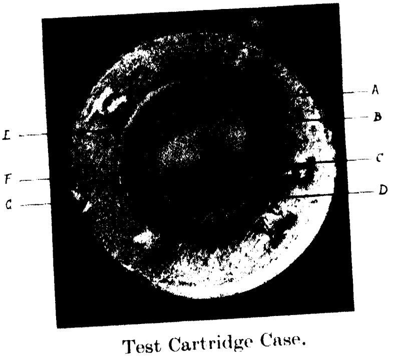


Fig. 6.



Crime Cartridge Case.



Test Cartridge Case.

Fig. 7.

examined microscopically is opaque and cannot always be subjected to any elaborate process of preparation, either because of its structure or because it may have to be presented in Court at a later period. Consequently the illumination of opaque objects is of considerable importance and it is found that the Heine Ultropak Illuminator is of great value when it is necessary to use incident light. No forensic laboratory should be without this very useful form of illuminator.

Finally it must be mentioned that photomicrography at times plays an important part in this work. Sometimes the result of an examination may have to be shown in Court and as already pointed out, this may be of particular value where two objects have been compared under a comparison microscope.

The use of the microscope to any great extent in forensic work is naturally of comparatively recent origin, and such use has coincided with the growth and development of forensic medicine which again has been dependent on the advances made in the application of the microscope to medical science as a whole. No doubt in the field of forensic medicine, the microscope was first of all employed in an attempt to identify blood stains. As is well known, microscopical examination of such stains, when completely dry, is of little value, but when they are moist or the blood is still in a coagulated condition, the individual red blood corpuscles can be readily observed. It was also used in the early days to differentiate the species from which the blood might have originated. The microscope is of little use in the identification of mammalian blood apart from that of the camel whose red corpuscles are oval in shape, those of the other species being of a roundish shape are similar to all intents in appearance and size, and it is extremely difficult or more or less impossible to get accurate measurements of their size, because of their partially dried condition. The blood corpuscles of birds, reptiles, and fish being oval and also nucleated are readily distinguished microscopically from what might possibly be thought to be human blood. As long ago as 1851, at the Essex Assizes, in the case of *Rex* v. *Drory*, " it was suggested by the defence that the blood stains on the clothes of the prisoner had been caused by his having killed some chickens. The shape of the corpuscles negatived this part of the defence. In another case the blood was alleged to be that of a fish ; this was disproved by the shape." A doctor was called on one occasion to see a patient who was spitting florid blood. On examination of the sputum with the microscope, he found the coloured blood corpuscles to be that of a bird. On his telling the patient that she had mixed a bird's blood with the expectoration, she was astonished and confessed that she had done so.* These cases illustrate the use that has been made with the microscope in the differentiation of red blood corpuscles in the past, but nowadays such a procedure is rarely adopted in an attempt to differentiate the species. Differentiation has now reached the highest degree of accuracy by means of

* Taylor, Principles and Practice of Medical Jurisprudence, 9th Edition. Vol. I, p. 476.

a serological method known as the precipitin test. Microscopical examination is essential in the chemical identification of blood stains. In both the specific tests for blood known as the Teichmann and Takayama tests, definite crystals are formed which can only be seen on microscopic examination.

In the spectroscopic examination of blood, the microscope is again essential where comparatively minute traces are being examined. This operation is carried out by means of the microspectroscope which can be attached to any monocular microscope. It is used in the identification of blood and as it is a comparison spectroscope, it is also of use in the identification of carbon monoxide hæmoglobin.

The grouping of blood is now a matter of considerable importance and in such agglutination tests, microscopical examination may be necessary in order to distinguish the clumping of the red blood corpuscles.

The definite recognition of seminal stains can only be carried out by means of the microscope. The position of the suspected stain may be seen by the unaided eye or with the help of the ultra-violet lamp, as such stains are usually fluorescent. However, a definite opinion cannot be given until spermatozoa are finally recognized. Small pieces of the stained fabric are soaked in acidulated water or normal saline solution and smears made from them by the usual method. After fixation they are stained by any of the various stains, hæmotoxylin and eosin being found to be the most reliable. It is interesting to note and valuable from the medico-legal point of view, that dried spermatozoa retain their form for a considerable period and can therefore be identified as such. They have been found in stains which were produced twenty-three years prior to examination. Spermatozoa, however, are not always readily found and occasionally considerable time and patience have to be spent in their search.

Hair may be of importance in certain medico-legal problems. The question may arise as to whether or not the hair is human. If human, from what sex and from what part of the body has it arisen, or could it have come from the head of a certain individual. This will involve careful comparison, preferably with a comparison microscope. In animal hairs the identification of the species will arise. This involves a careful study of the medulla, cortex, and cuticle. The appearance of the cuticular scales has recently been shown to be of value for identification purposes, as they vary in shape, size, and number in the different species. They can be examined by means of oblique illumination or casts of the hair may be taken in a preparation of collodion or any of the various nail varnishes, when the cuticular scale impressions will be readily seen with a $\frac{1}{6}$-in. objective. The medulla may also be distinctive and in heavily pigmented hairs, bleaching may be necessary before it can be observed. The colour and appearance of the pigment in the cortex is naturally of importance. In addition, it may be necessary to study the shape of the hair, and in order to do this, transverse sections must be made and mounted for microscopical examination. Occasionally a hair may reveal evidence of the infliction of violence or other information,

such as the period of time which has elapsed since it was cut or whether it had been permanently waved or dyed.

Microscopic examination of documents in cases of forgery or fraud is comparatively simple but important work, as frequently erasures and changes of a fraudulent character are present, which are quite invisible to the naked eye, but become very obvious under the microscope. In this type of work, the Greenough stereoscopic microscope is of the greatest value, as it is better not to use too high magnifications. There is the examination of the surface of the paper for evidence of erasures, followed by examination of the writing itself, as for example in a forged signature. Such signatures may be laboriously drawn from a real signature with frequent lifting of the pen and occasional stoppage of its movement, even when not lifted. In these cases the uneven distribution of the ink and irregularity of the lines can readily be seen. When writing is traced by a pencil or carbon paper and then inked over, uncovered portions of the tracing may still be seen, or there may be obvious particles of graphite mixed with the ink. Fraudulent additions to writing may be clearly seen by the different appearances of the inks and also from the appearance of the crossed strokes ; those which were last written being discernible as such. Although inks may have to be examined chemically, microscopical examination may show at once that one ink has a sediment in it which may not be present in another type. Again, it may be ascertained by careful examination whether a document was written on before or after it was folded. In fraudulent type-written documents, microscopical examination may prove to be of great value. In all cases of documentary work good photomicrographs showing evidence of fraudulent alterations are necessary, in order to present the case clearly to the Court.

At times a careful investigation of the composition of the paper may be necessary, in addition to the study of the writing and ink. As is well known, papers may be made from different fibres such as linen, jute, manilla hemps, or wood pulps. Microscopical examination will reveal the character of the paper, and it may be shown for example, that a document which was supposed to have been written in a certain year could not have been as the type of paper on which it was written was not in use at that time.

The examination of dusts and fibres which may be found in relation to certain criminal activities is, at times, of considerable importance. This involves the identification of such dusts and fibres and their comparison with known samples, which may possibly prove to be a connecting link between a suspect and the perpetration of the crime. An example of this is found in the case of a criminal who tears one of his garments at the scene of a crime ; if a fibre or fibres, found later adhering to some object, prove to have similar characteristics to those from his coat, then the fact is highly suggestive of his implication in the case, especially when considered with other evidence which may be at hand.

As has already been indicated micro-comparison work is of considerable importance in its forensic applications. The following examples will help

to indicate this point. In the case of forcible entries where some tool has been used, such a tool being commonly known in criminal circles as a jemmy, scratches or marks are reproduced on the woodwork, paint, or metal at the point of entrance. The edge of the jemmy may have a characteristic shape or may be chipped so that the position of the marks will correspond to test scratches made by the suspected tool. Not only will their relative positions be similar, but on magnification it will be seen that the finer or secondary scratches are also similar.

The microscope has within recent years brought the identification of fire-arms to the level of an exact science. Formerly it could be said that a bullet could probably have been fired by a weapon of a certain make, but it was only by mere supposition that the bullet could be tied down to a definite firearm. There were always witnesses, as there always will be, who attempted to impress upon the judge and jury their opinions and render them as definite facts without having any accurate scientific proof. By the use of the comparison microscope it can now be definitely proved that a bullet or cartridge case was, or was not fired by a specific weapon. Briefly this is done by a comparison of the bullet and cartridge case found in relationship to the crime with those which have been experimentally fired from the suspected weapon. In the case of automatic pistols, revolvers, and high velocity rifles, what are known as lands and grooves, which form the rifling of the barrels, produce indentations on the bullet. Finer scratches or secondary markings are present on these lands or ridges and grooves which are in turn reproduced on the bullet as it passes along the barrel of the weapon. It is by the micro-comparison of these finer scratches that it can be definitely proved that a certain weapon fired a certain bullet. Cartridge cases can also be similarly used for identification purposes by a comparison of the microscopic scratches chiefly found on the soft metal cap and base of the case. On the occurrence of the explosion in the chamber of the weapon there is a recoil of the cartridge case against the posterior part of the chamber which is known as the breach-block. As this has been smoothed by filing, the microscopical file marks will naturally still remain. These or any other marks which may be there are reproduced by the force of the recoil on the base of the cartridge case and can be readily compared by means of photomicrographs. Micro-comparison of the marks in the depression on the cap which is produced by the firing pin are also of value.

The microscope in its different forms has thus been brought more and more into contact, during the last decade, with the solution of problems in forensic science, and it will be seen that considerable advance has been made and will continue to be made, as our knowledge increases. It is interesting to note how the field has spread beyond its original boundary of forensic medicine into a much wider field embracing all branches of what has now been termed forensic science.

DESCRIPTION OF PLATES.

Plate I.

Fig. 1.—Edge of jemmy showing small fragment found at scene of crime and which fits into edge of instrument subsequently found.

Fig. 2.—Jemmy showing irregular edge with scratches on lock which obviously correspond to indentations on the instrument.

Fig. 3.—To be compared with fig. 2. (*a*) shows original marks on lock which correspond with (*b*) marks experimentally made with instrument shown in fig. 2.

Fig. 4.—Higher power magnification of scratches in marks from fig. 3, and showing that correspondence.

Plate II.

Fig. 5.—Comparison photomicrograph of (*a*) counterfeit coin showing flaws with (*b*) genuine coin.

Fig. 6.—Comparison photomicrograph of two similar hairs.

Fig. 7.—Comparison photographs of two cartridge cases showing similarity in markings on the bases of both cartridges.

78. 31. # VII.—INCREASING THE DEPTH OF FOCUS IN PHOTOMICROGRAPHY BY INCIDENT LIGHT.

By Walter Koch, M.D., Ph.D.

(Hebrew University, Jerusalem.)

One Plate.

When using transmitted light in photomicrography it is possible to increase the depth of focus considerably by stopping down the condenser diaphragm. This method, however, is not applicable in photomicrography by incident illumination, since in this case a reduction of the angle of illumination does not produce any relevant effect.

A success can be attained by another method. By inserting a stop immediately above the object glass the depth of focus can be considerably increased. The procedure will of course diminish the resolving power, but at the same time correct the errors of the lens and, therefore, the sharpness of the picture will not be remarkably affected provided the stop is closed down within moderate limits.

In the following a method is described by which a considerable increase in depth of focus can be attained by simple means.

It is recommended to use a set of three circular thin plates with a central hole. The external diameter of these plates is the same as the diameter of the fixed diaphragm of the object glass. The diameter of the holes is 1, 2, and 3 mm. respectively. The stops are either put direct on the object glass diaphragm or fastened to an intermediate piece which guarantees the exact central position of the aperture of the stop. The choice of the right stop is determined by the wanted depth of focus and the necessary resolving power.

There are two ways of determining the most suitable aperture of the stop:

(1) The right aperture is found by focussing visually through the microscope or by test exposures of the object to be photographed. This method, however, often proves unsatisfactory and, therefore, the following general method is advocated.

(2) *Principle of the method.*—A stage micrometer is photographed in transmitted light. This allows us to measure directly the increase in depth of focus and to come to a very close estimate of the loss of resolving power.

Execution.—(*a*) A stage micrometer is viewed in transmitted light with *open* substage diaphragm. If a set of eyepieces is available, the one is

Fig. 1.

Fig. 2.

Fig. 3.

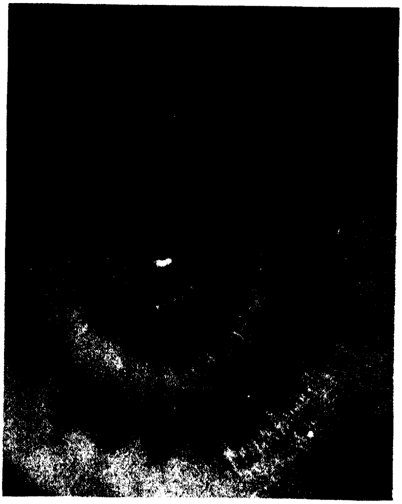


Fig. 4.

[To face p. 86.

chosen which produces the best flatness of field. If the flatness of field is insufficient, i.e., if towards the periphery the division marks become blurred, the stage micrometer is photographed in the horizontal position.

(*b*) One end of the stage micrometer is lifted about 2 cm. by placing a cover-slide box underneath, and then the middle of the stage micrometer is focussed again. In this position both ends of the scale appear out of focus, as they are lying in a higher or lower plane respectively than the middle. Now another exposure is made.

(*c*) Without changing the oblique position of the stage micrometer, the stops are inserted one by one above the object glass, and the stage micrometer is photographed through each stop.

In this manner five different negatives are obtained: one of the stage micrometer in horizontal position and four of the same in inclined position. The depth of focus is determined by counting with the aid of a magnifier the number of division marks which appear sharp. The narrower the stop the more division marks will appear sharp. If the height of the box is 20 mm., the length of the slide is 76 mm., and the length of the stage micrometer itself 1 mm., the height of the stage micrometer is

$$20 : 76 = \times : 1$$
$$\times = 0{\cdot}26 \text{ mm.}$$

Thus, if the stage micrometer is divided into 100 marks, each mark has a value of height of 0·0026 mm.

The gradual decrease of the resolving power with the restriction of the bundle of light leaving the objective lens can be tested by examining the central division mark. Though focussed exactly, it becomes more and more unsharp the smaller the hole in the stop.

To facilitate the comparison of the different pictures, a print of each should be made. When cutting these prints lengthwise in an angle perpendicular to the division marks, the corresponding marks can be easily compared and the effect of the different stops determined.

Summary.

A method is described to increase by simple means the depth of focus in photography by incident light. The effects on the depth of focus and the resolving power are discussed.

DESCRIPTION OF PLATE.

Fig. 1.—*Helicella sp.* Without stop. ×30.
Fig. 2.—*Helicella sp.* The same arrangement. Diameter of hole in stop 3 mm.
Fig. 3.—*Helicella sp.* The same. Diameter of hole 2 mm.
Fig. 4.—*Helicella sp.* The same with diameter of hole 1 mm. This figure shows a remarkable loss of definition due to the decrease of resolving power. By the narrow bundle of rays, bubbles in the optical system are now depicted as small spots. In spite of the lack of definition the depth of focus has increased when compared with fig. 3.

5.826.1.

VIII.—ON CHLORAZOL BLACK E AND SOME OTHER NEW STAINS.

By H. Graham Cannon, Sc.D., F.R.S., Beyer Professor of Zoology in the Victoria University of Manchester.

In 1937 I described Chlorazol Black E as a new biological stain for general purposes. This has been confirmed recently by Darrow (1940) who emphasizes the ease of its application in plant tissues as well as animal. Also Nebel (1940) in the same journal states that in cases where the acetocarmine method of demonstrating chromosomes is not satisfactory a preliminary treatment with chlorazol black produces very sharp staining effects. He also states that the stain has been "put on the certification basis" by the Commission on the Standardization of Biological Stains, Geneva, N.Y. Both Darrow and Nebel quote Eric Black EXOO and Pontamine Black E as being synonyms of Chlorazol Black E.

Dobell (1938) has found an interesting use for the stain in the identification of cysts in films of intestinal amœbæ. He showed that while the nuclei and chromatoid bodies in the cysts of Entamœba stain a very sharp black, the cyst walls appear greyish-green, while the glycogen in the cysts stains red. He states that it takes 1–1½ hours to produce this effect, which is considerably longer than that for ordinary sections, but it is the only method known to him that will stain all the structures at once and with a single solution.

In correspondence, Mr. F. S. Russell, F.R.S., tells me that he uses the stain regularly in his cœlenterate studies as it stains very clearly the delicate perisarc which houses the hydroids. We have confirmed this in ordinary class work and have been able to demonstrate the pattern along the edge of the hydrotheca of Obelia. We also find it very useful in selectively staining the nematocyst batteries in such things as the ephyræ of Aurelia.

Le Page has used chlorazol black for staining nematodes, and it was he that discovered that dilute "Milton" can be used for taking out the stain from overstained specimens.* I find that it is possible, and sometimes advisable, to overstain solid pieces of tissues or whole specimens and then remove the excess of stain gradually. However, I have never been able to overstain thin sections. In my experience the sections stain in about half an hour, and then even if left in the stain for weeks do not overstain.

* I have to admit an inexcusable mistake in my letter to "Nature" (1937). I stated there, for some unaccountable reason, that stained sections can be differentiated in terpineol. It should have been *pyridin*, not terpineol.

J. P. Harding has described in a previous number of this journal (1939) how he dissects minute crustacea in benzyl alcohol. He finds that, in the case of very small Ostracods, the chlorazol black with which he has previously stained the whole specimen becomes partly washed out while he is manipulating the extremely minute isolated limbs. He therefore carries out his final dissection and manipulation in benzyl alcohol saturated with chlorazol black—a solution which looks like black ink but which in thin layers appears pale and transparent.

J. R. Baker (1941) has recently shown that chlorazol black can be used as a vital dye. Injected subcutaneously into living mice it circulates in the blood and shows an affinity for the reticulo-endothelial system. Since it is not excreted the latter tissue can be loaded to capacity and sections of material fixed in Zenker show up the black particles of the dye in the cells which have absorbed them in a very striking manner.

For zoological class work we find the stain invaluable. Our routine method for all such standard material as Obelia, Echinoderm larvæ, Polyzoa, Tunicates, etc., all of which is invariably put out to the class in 70 p.c. spirit, is as follows :—transfer to chlorazol black saturated in 70 p.c. spirit for 20 minutes—wash away excess of stain in 90 p.c. spirit—leave in this strength of spirit for some time according to the thickness of the specimen—transfer to benzyl alcohol. This clears the specimen and it can be mounted direct into Canada balsam. The only real drawback to the method is that it is too simple and does not provide a sufficient test of the student's ability to make a permanent preparation.

As regards permanence it is now 4 years since I discovered chlorazol black as a biological stain, and my first preparations show no signs of fading. I have noticed, however, that stained specimens left in benzyl alcohol in daylight are apt to fade quite rapidly. This may be due to the fact that the benzyl alcohol used for the clearing may hold a considerable proportion of water. I therefore make a practice in critical preparations of washing away the benzyl alcohol with xylol before mounting in balsam.

For photomicrography I find the colour of chlorazol black preparations very good. As an example I refer to the plates so beautifully reproduced for me by the "Discovery" Committee in my report on Gigantocypris (1940). These are from negatives taken on Ilford Chromatic Plates—there is no need to use Panchromatic—through a pale yellow-green filter (Ilford Micro 8).

I have recently been investigating the possibilities of chlorazol black as a routine botanical stain and discovered that its staining effects are influenced markedly by the solvent. In ordinary ethyl alcohol it stains cell walls black and other cell structures various colours from yellow through greenish-grey to black (see Darrow, 1940, p. 66). In benzyl alcohol, however, it stains selectively the pits in the cell walls. In a longitudinal section of the stem of Helianthus the wall of a pitted vessel appears as a sheet of black or dark grey dots on a colourless background. This effect is, how-

ever, best seen in sections of conifers where the bordered pits are picked out by the stain. In a longitudinal section of the stem of *Pinus sylvestris* each bordered pit appears as a grey circular patch representing the torus surrounded by a concentric grey ring which indicates the margin of the pit cavity. This staining effect is not rapid and hand sections should be left in the stain for an hour or so, but again, overstaining appears impossible and so sections, if convenient, can be left overnight in the stain.

Another use especially valuable for ordinary botanical class work is in the staining of macerated tissues. Specimens can be macerated in the form of thin slices overnight in 1 p.c. chromic acid. I have tested preserved material of the artichoke stem and the wood of lime and pine, and find that the isolated cells stain very readily with chlorazol black in benzyl alcohol. With careful handling thin slices after maceration can be dehydrated into benzyl alcohol before disrupting them. If they are then teased in a drop of chlorazol black in benzyl alcohol the cells will be seen to take up the stain in a matter of seconds. They appear on the whole a dark bluish-grey and do not readily overstain. The large pitted vessels are particularly clear while the spiral bands of thickening on the walls of the inner vessels stain a sharp black. An interesting effect is produced in the wood fibres where the slit-like pits stand out clearly. In a preparation of poplar they appear as a cross on a dark grey background with one arm sharp and black and the other less distinct and pale.

Lignin Pink.

In some of my earliest preparations of the artichoke stem I noticed that the lignified cells stained the faintest pink. The dye responsible for this has been isolated for me by Imperial Chemical Industries. It is an impurity occurring normally in minute traces in chlorazol black E. I have tested it by comparison with other recognized methods and find that it is specific for lignin and am therefore calling it Lignin Pink.

It is a monazo acid dye. A saturated aqueous solution stains the lignified tissues a deep carmine in about 20 minutes and, provided the sections are normally thin, leaves all the other tissues completely unstained. Sections left in the solution for days produce no further effect beyond a slight increase in the intensity of the red colour. In alcoholic solution it appears of little value with plant material as it stains all tissues pink in varying degrees.

A satisfactory *simple* double stain for ordinary plant tissues is badly needed for routine elementary class work. The staining of a hand-cut section of botanical material is a standard question in elementary practical examinations, and a double stained preparation is expected by the majority of examiners. The usual methods adopted by students are capricious and their results do not always afford evidence of the skill of the student using them. Such a simple solution actually exists in chlorazol black, but the pink stain is much too faint. I therefore increased the amount of lignin pink and found the single solution absolutely satisfactory.

I have tested various concentrations of lignin pink and find that the exact proportion is not important and can be varied according to taste. A solution of equal parts by weight of Chlorazol Black E and Lignin Pink in distilled water is a useful standard. I use 0·5 grm. of each stain in 100 c.c.s. of water. This will give an excellent double effect in about 20–30 minutes. The lignin stands out bright carmine against the surrounding black stained tissues. As long as the sections are reasonably thin the results are constant. The stain does not wash out in alcohol and overstaining seems to be impossible. Since neither mordanting nor differentiation are necessary and the results do not fade this single solution double stain appears to satisfy all the requirements an elementary student might desire.

As a stain for animal tissues lignin pink is practically useless for sections while it is excellent for staining whole organisms or isolated pieces. Sections left for days in the stain refuse to take it up while a medusa of Obelia or an isolated limb of a crustacean will stain a deep carmine after about 15 minutes in aqueous solution or a little longer in alcoholic. In this it resembles borax carmine.

With cœlenterate material it is similar in its action to Chlorazol Black E in that it stains the perisarc uniformly and at the same time picks out the nematocysts.

It is especially useful as a chitin stain. It will stain the finest structures on an ostracod appendage a uniform pink in 15 minutes in aqueous solution. A better effect, however, can be obtained by using a solution of the dye in benzyl alcohol and leaving the specimen considerably longer—sometimes overnight staining is necessary. The final result in this case is a definite purple for the exoskeleton, while the other tissues are more a carmine.

Arising out of these further studies of Chlorazol Black E I have obtained through the courtesy of Imperial Chemical Industries a new series of stains, all of which are just as permanent and as simple in their use. In testing them I have used the type of material one would expect to find in an ordinary biological laboratory. For animal tissues I have used sections of late tadpoles fixed in B. G. Smith's fixative and of Gammarus fixed in alcoholic Bouin. For plant tissues I used stems of the Jerusalem artichoke and the lime tree fixed in spirit for ordinary class work.

The stains * can all be described as similar in their method of use and in their general effect to Chlorazol Black E, but of a different colour. They stain nuclei and chromosomes deeply and very sharply while the cytoplasm stains the same colour but faintly. None of them require a mordant and none differentiation. More important, with the exception of Owen's Blue, I find it practically impossible to overstain sections. For the sake of convenience I have used saturated solutions, either aqueous or in 70 p.c. alcohol,

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* I have named these new dyes after Profs. A. Milnes Marshall, F.R.S. and S. J. Hickson, F.R.S., who both occupied the Beyer Chair of Zoology at Owen's College, now the Victoria University of Manchester.

and find that staining is effected in about 20 minutes. I have tested all the stains for the last two years or more and find no trace of fading.

Hickson Purple.

This is a disazo dye belonging to a class which is not represented among commercial dyes of known structure.*

It gives a very sharp purple effect in aqueous solution. An interesting double effect is produced as, while leucocytes stain purple like the rest of the tissues, the red corpuscles stain a distinct red.

The dye is of no value in alcoholic solution.

Marshall Red.

This is a disazo dye which belongs to the J-acid urea class of direct dyes.

It produces a red effect nearer vermilion than carmine in aqueous solution. It also gives a double effect as it leaves the red blood cells unstained while staining the other tissues, including leucocytes, red. The real value of this stain is obtained in combination with Victoria Green (see below).

It is of no use in alcoholic solution.

Beyer Brown.

This is a disazo dye belonging to the benzidine class.

It can be used in either aqueous or alcoholic solution and produces an effect very similar to a really good Ehrlich's hæmatoxylin.

Plant tissues stain very sharply and companion cells stand out remarkably well.

Victoria Green G.

This is a trisazo dye which belongs to the benzidine class of direct cotton dyes.

This gives markedly different effects when used in aqueous and alcoholic solutions. The solution in water produces a useful blue-green result, while in alcohol the general effect is a pale yellow-green. The latter is not of much use but shows an interesting double effect. The red blood cells stain the typical blue-green of the aqueous solution and show up in marked contrast to leucocytes and other tissues. Thus a single erythrocyte in a capillary of the brain stands out very clearly from the surrounding nerve cells. In aqueous solution the red cells remain practically unstained while the leucocytes stain blue-green like the other tissues.

In arthropod material the chitin appears to react in the same way as blood cells. In aqueous solution it remains unstained while in alcoholic it stains a blue-green.

In plant material the green stain taken up from an aqueous solution

* I have to thank my colleague Dr. William Bradley for the chemical descriptions of these dyes.

washes out subsequently in the alcohols, but in alcoholic solution a double effect is produced as the lignin stains blue-green against the surrounding yellow-green effect.

Manchester Blue.

This is a disazo direct cotton dye and belongs to the benzidine class.

It can be used in either aqueous or alcoholic solution and gives a very sharp dark blue effect. In alcohol, however, the staining takes much longer —the specimen should preferably be left overnight in the stain.

Owen's Blue.

This is a disazo dye similar in constitution to Manchester Blue.

It can be used in either aqueous or alcoholic solution and gives a violet-blue effect. It is very powerful and the only one of this series that tends to overstain. It is best used in alcoholic solution when a good result is obtained in the usual 20 minutes. The saturated aqueous solution, however, must be diluted to 20 p.c. of this strength.

The aqueous solution produces a general purple effect with plant tissues in which the lignin stains a pale but definite pink. In alcoholic solution, however, the lignin stains a deep blue, the remaining tissues being practically unstained.

Combinations of the above Stains.

From their similarity in general staining reactions it is clear that it would be unlikely that these new stains would lend themselves generally to double staining effects—they are all essentially nuclear stains. However, the peculiar nature of Victoria Green in that its alcoholic and aqueous solutions yield markedly different results suggested that it might lead to a useful combination with other stains. I tested this and found that, combined with Marshall Red or Hickson Purple, really good double staining effects can be obtained.

Marshall Red and Victoria Green.

Stain sections in a saturated aqueous solution of Marshall Red for 20 minutes—rinse in distilled water—stain in saturated 70 p.c. alcohol solution of Victoria Green for 30 minutes—rinse in 90 p.c. alcohol, dehydrate and mount in the usual way.

In successful preparations of animal tissues the myofibrils stain a sage green while the nuclei appear a bright carmine. The results vary somewhat, but always the muscle fibres appear greenish to greenish-grey while the nuclei are red. The white matter of the spinal cord stains yellowish-green while the cartilage appears pink. The red blood cells are practically unstained. The retina stands out remarkably well as the rods and cones appear bright bluish-green.

Hickson Purple and Victoria Green.

The method is the same as for the Marshall Red combination, but the time of staining can be decreased for the purple to 10 minutes and increased for the green up to 1 hour.

This method produces an improvement on the use of Hickson Purple alone. The vividness of the colour is toned down and the nuclei appear even more sharply defined. The double staining effect is not very marked in the muscular tissues; it is in the red blood cells that this stands out, for these stain a vivid green and so contrast markedly against the general blue-purple surroundings. The results do not vary much and I find this combination very useful for ordinary class work demonstration sections.

Note:—All the dyes mentioned in this paper may be obtained from British Drug Houses, Ltd.

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ABSTRACTS AND REVIEWS.

ZOOLOGY.

(*Under the direction of* G. M. Findlay, M.D.)

HISTOLOGICAL TECHNIQUE AND STAINING.

A Phenol-Thionin Stain for Nervous Tissue.—G. C. Grant ("A Formalin-phenol-thionin Stain for Nervous Tissue," *Stain Technol.*, 1941, **16**, 125–6). Nervous tissue fixed by perfusion with 10 p.c. formalin and stored in this reagent stains very lightly in 1 p.c. aqueous phenol-thionin, but with the following modifications staining is satisfactory: wash nitrocellulose sections in distilled water; stain for 15–30 minutes at 50° C. in a mixture of 0·5 p.c. aqueous thionin (C.I. 920) 25 c.c., 2 drops of liquefied phenol, and 2 drops of formaldehyde (34-38 p.c U.S.P.); rinse in distilled water; wash in 70 p.c. ethyl alcohol; place in 95 p.c. alcohol for 2–4 minutes, absolute alcohol plus 12–15 p.c. of U.S.P. chloroform; clear and differentiate sections individually 1–10 minutes in equal parts of oil of origanum cretic; oil of bergamot, f.b. extra-fine; phenol; place on clean glass slide with forceps; roll sections flat with a camel-hair brush; blot dry with bibulous paper; wipe round sections with a cloth moistened in xylol to remove oil droplets; remove any lint or dirt with a clean, dry camel-hair brush; mount in damar, clarite, or balsam. G. M. F.

Fading in Mounting Media.—R. D. Lillie ("Stain Fading in Various Histologic Mounting Media," *Stain Technol.*, 1941, **16**, 127). Sets of sections of monkey pancreas, adrenal, spleen, kidney, and heart were stained either with Weigert's iron chloride hæmatoxylin and Van Gieson's picrofuchsin with 0·1 gm. fuchsin per 100 c.c. saturated aqueous picric acid or with buffered Romanowsky stain at *p*H. 4·2. One set of sections with each of the stains was mounted in neutral Canada balsam in xylol; one set in Curtis' salicylic acid balsam; one set in 60 p.c. clarite in xylol; one set in 60 p.c. clarite X in xylol; and the last set in heavy liquid petrolatum U.S.P. ($N\triangle=1{\cdot}483$), and sealed with pyroxylin cement. The pancreas and adrenal sections were exposed under a 100-watt electric light at a distance of 22–25 cm. The others were left on a north window-sill in diffuse daylight for 6 months. Clarite was the most useful mounting medium for the two stains. Liquid petrolatum preserves Romanowsky staining, but cover glasses must be cemented, and often leak, thus creating an oily film not only on the adjacent part of the slide but over the margins of the cover glass. G. M. F.

The New Electron Microscope.—H. E. Rhea (*Science*, 1941, **93**, 357–8). The first electron microscope was bulky and so complex that it required the services of an electronic engineer to operate it. The new model fits into a small room and is far easier in operation. Including its power supply, the microscope is contained

in one rack 7 ft. tall and weighs about 500 lb.; a normal 110-volt line socket supplies the current. G. M. F.

An Improved Micrometer.—J. C. LOTZE and M. J. YIENGST ("An Apparatus for measuring Microscopic Objects," *Science*, 1941, **93**, 45–6). A device is described for measuring objects less than 1μ in diameter. The apparatus comprises a microscope, the prism of a camera lucida, and a screen. The latter covers a lighted box pierced with calibrated holes and slits that represent, as viewed through the microscope, established lengths with which the size of microscopical objects can be compared. G. M. F.

Fluorescence Microscopy in Biology.—P. ELLINGER (*Biol. Rev.* 1940, **15**, 323–50). This is a comprehensive review of the subject, both from the historical and instrumental aspects. The history does not go further than the earliest observations made by Kohler, of Jena, in the development of microscopy with ultra-violet light. It is known that fluorescence is one of the greatest difficulties inherent in all ultra-violet work with the microscope; in fact, it is probable that such work is the best possible training for a complete understanding of the possibilities and limitations of fluorescence microscopy. Kohler recognized that it would be possible to make use of the fluorescence of the objects that he observed and that the interpretation of such images might form the basis of a new method of research. Under suitable conditions there is no doubt the fluorescent image is as perfect as the microscope can yield: the ideal condition of self-luminosity is achieved. The essential feature of any equipment for such work is the provision of a suitable source of ultra-violet light, and the carbon arc is still among the most suitable as well as the least costly. About 1912 apparatus was designed by Zeiss and Reichert, the former using transmitted light and the latter dark-ground illumination, on which little improvement in principle or in detail is observable in recent appliances. Any recent advances are dependent rather on the introduction of light sources in which the output of ultra-violet light is increased, such as in mercury arcs of high intensity. A more recent development is the use of fluorescent dyes, these having a selective action, as in the staining processes in use for ordinary microscopy. In this case the fluorescent reaction is in terms of the response of the dye itself to ultra-violet light and not that of the tissue or object which absorbs the dye. As in ordinary staining, the result is also dependent on the elimination of the dye from certain elements of structure so that only the desired portion is fluorescent. The paper gives a fairly full account of some recent work, together with a bibliography of considerable value and extent. J. E. B.

Staining the Nucleolus.—C. S. SEMMENS and P. N. BHADURI ("Staining the Nucleolus," *Stain Technol.*, 1941, **16**, 119–20). As fixatives, chrom-formalin mixtures give good results; but any fixative is suitable provided before hydrolysis the material is left in 1 p.c. chromic acid solution for a few hours, thoroughly washed in water, and left over-night in 75 p.c. alcohol. Sections are taken down to water in the usual way, except that they are left in 75 p.c. alcohol for at least 2 hours. After washing in warm water and leaving for abo ut 5 minutes, sections are placed in 12 p.c. hydrochloric acid at 60° C., on the a verage about 20 seconds. The staining jar is removed from the oven and immersed in a vessel of cold water. When cold the used acid is poured off and the slides covered with fresh cold N HCl; this is poured off and covered with decolorized fuchsin for 3 or 4 hours or over-night. The dye is then poured off and the slides covered with bleaching solution, using two changes of this with 30 minutes in each. Rinse in distilled water, in 50 p.c. alcohol, then 70 p.c. alcohol, and place for at least 1 hour in a

mordant of 80 p.c. alcohol saturated with sodium carbonate. The mordant is poured off, and sections are quickly rinsed in 95 p.c. alcohol, then for 20–25 minutes in a light-green solution (a saturated alcoholic solution of light green S.F.Y. (C.I. 670) is filtered), and 2–3 drops of pure colourless anilin oil are added per 100 c.c. of dye. The dye is drained off and the slide rinsed in a differentiating solution of saturated sodium carbonate solution in 80 p.c. alcohol 10 c.c.; 80 p.c. alcohol 90 c.c. Differentiation is continued in 95 p.c. alcohol under the microscope, if necessary returning to the differentiating solution, and then back to the 95 p.c. alcohol. Green is removed except from the nucleolus. Dehydrate in two or three changes of absolute alcohol and clear in alcohol-xylol (50–50), xylol-alcohol (75–25), followed by pure xylol. Mount in " Sira " or other neutral medium. G. M. F.

Clearing Tissues with Organic Phosphates.—R. A. GROAT (" Clearing Tissue with Mixtures of Tributyl and Tri-*o*-cresyl Phosphates," *Stain Technol.*, 1941, **16**, 111–17). Mixtures of tributyl phosphate and tri-*o*-cresyl phosphate of varying proportions may be used as immersion fluids for clearing gross specimens. By varying the ratio of the former to that of the latter (measured volumetrically) from 1 : 130 to 22 : 110, mixtures having refractive indices varying from 1·555 to 1·534 can be made. A table of mixtures and refractive indices is given. Preparation of specimens consists of skinning, eviscerating, fixing, washing, bleaching when necessary, dehydrating, and defatting. The proper clearing mixture is determined by the immersion method, which entails microscopic observation of a 50μ section of tissue to be cleared. G. M. F.

Chlorazol Black E.—N. D. LEVINE and C. C. MORRILL (" Chlorazol Black E. A Simple Connective Tissue Stain," *Stain Technol.*, 1941, **16**, 121–2, 2 text-figs.). Cannon's finding that chlorazol E gives sharp differentiation in histological and cytological preparations is confirmed. A 0·5 p.c. solution in 70 p.c. alcohol is saturated; a staining period of 30 minutes was found to give better results than shorter times. G. M. F.

Micro-incineration of Frozen Sections.—W. T. RODDY (" Frozen Section Micro-incineration," *Stain Technol.*, 1941, **16**, 101–104, fig. 1). Frozen sections can be subjected to micro-incineration. Material containing diffusible or soluble salts is cut on the freezing microtome. No fixation is used, but if the salts are not soluble in formalin the tissues are placed in a 10 p.c. aqueous formalin solution. Sections are cut at 20μ, and if soluble salts are present, transferred at once to xylol and mounted on a Corex D slide, already coated with a glycerin-gelatine film; then flattened by pressing on the slide with filter paper. If the salts to be demonstrated are insoluble the frozen sections are dehydrated in 50, 70, and 95 p.c. ethyl alcohol, and mounted as before. Sections are incinerated in an electric furnace and the temperature of incineration is dependent on the type of tissues to be incinerated and the character of the salts present. If no fixation is required the whole procedure can be carried out in 1 hour. G. M. F.

Adsorption of Dyes by Bacteria.—T. M. McCALLA and F. E. CLARK (" Dye Adsorption by Bacteria at Varying H-ion Concentrations," *Stain Technol.*, 1941, **16**, 95–100). Adsorption of hydrogen ions and dye cations by washed bacterial cells shows a reciprocal relationship. H-ions and crystal violet ions are held by the cell at the same adsorption centres and the influence of $H+$ on basic dye adsorption is one of direct competition or replacement. The adsorption of $H+$ and acid fuchsin is similar in that an increase is noted as the pH of the suspension is lowered. G. M. F.

Demonstration of Hæmoglobin.—P. H. Ralph ("The Histochemical Demonstration of Hemoglobin in Blood Cells and Tissue Smears," *Stain Technol.*, 1941, **16**, 105–6, 1 text-fig.). A method for demonstrating the presence of hæmoglobin in cells by a specific staining reaction is described. An ordinary dried blood smear is flooded with a 1 p.c. solution of benzidine in absolute methyl alcohol and allowed to stand for 1 minute. The benzidine solution is then poured off and replaced with a 25 p.c. solution of superoxol in 70 p.c. ethyl alcohol. This is allowed to stand for 90 seconds, then washed in distilled water for 15 seconds, after which the smear is dried and mounted in neutral Canada balsam. All structures containing hæmoglobin are coloured dark brown. In the living erythrocyte of frog blood the hæmoglobin is all in the cytoplasm. To study other blood cells the preparation may be stained with Wright's stain. G. M. F.

An Inexpensive Lamp.—R. E. FitzPatrick ("An Inexpensive Lamp for the Dissecting and Research Microscope," *Stain Technol.*, 1941, **16**, 107–109, 2 figs.). The source of light is a 60-watt bulb with a silvered neck: a glass rod 5 in. long by $\frac{7}{16}$ in. diameter carries the light from the surface of the bulb to the object to be illuminated. To provide an even field of light the end surfaces of the rod are ground flat on a carborundum wheel and finished with emery or fine carborundum powder. The container is made from a tin, 4 in. diameter by $6\frac{1}{2}$ in. deep. The top of the tin is cut away and a hole to take the glass rod is made in the bottom, with a collar $1\frac{1}{4}$ in. long of galvanized iron or sheet brass soldered in the hole to hold the rod in position. For ventilation a rectangular opening, 1 in. by $1\frac{1}{4}$ in., is cut lengthwise in the underside of the can near the bottom. Some adjustment of light intensity is obtained by moving the rod in towards or away from the surface of the bulb. For critical work, light filters and diaphragms may be affixed to the end of the rod. G. M. F.

Microbial Action on Fats.—C. H. Castell ("*p*-Aminodimethylaniline monohydrochloride as an Indicator of Microbial Action on Fats," *Stain Technol.*, 1941, **16**, 33–6). By examining the action of *p*-aminodimethylaniline on triglycerides, fatty acids, and other organic compounds, it was shown that the dye in the reduced condition had little effect on fats, but was readily absorbed and oxidized by the fatty acids, producing shades of colour of light brown to black. As the dye becomes oxidized it becomes increasingly soluble in fat and fatty acids. Agar plates containing fat emulsions were flooded with the dye and inoculated with pure cultures of 39 different species of bacteria; varying colour changes were noted in the fat globules. G. M. F.

An Embedding Method.—C. E. Woodworth ("A Useful Embedding Technic," *Stain Technol.*, 1941, **16**, 124). The method is suitable for use with insect or other tissue. After dehydrating and infiltrating in the usual manner the embedding paraffin and the ends of a pair of forceps are heated over boiling water to 180° F., or more. An embedding boat is filled with hot paraffin and placed on an ice cube to cool. When the lower part of the paraffin is completely opaque lift the tissue by means of the hot forceps from the infiltration chamber to a dish of hot paraffin for an instant or until the cool paraffin around the tissue is melted; then transfer to the embedding boat. Orientate and allow to cool. G. M. F.

Numbering Celloidin Sections.—J. M. Hamilton ("A Method for Numbering Serial Celloidin Sections," *Stain Technol.*, 1941, **16**, 125). Sections are cut and placed chronologically between slips of paper in the usual manner. An "ink" is prepared by thoroughly mixing equal parts of 20 p.c. nitrocellulose (Hercules Powder Co., RS $\frac{1}{2}$ sec.) in amyl acetate and black oil colour (e.g. Martini

Studio Oil Colour, Ivory Black). One corner of each section is blotted and then numbered by means of a small camel-hair brush. Before returning the sections to the alcohol, the ink is " fixed " with a drop of chloroform applied with a second brush. G. M. F.

The Temperature of the Microtome Knife.—J. W. DUFFIELD (" Temperature Control for Microtome Knives," *Stain Technol.*, 1941, **16**, 123–4, 1 text-fig.). An apparatus for chilling or warming a microtome knife by means of water circulation is described. A water-jacket consists of two pieces of $\frac{1}{32}$-in. sheet copper soldered together and two nipples of $\frac{3}{8}$ or $\frac{1}{4}$ in. copper or brass tubing for the attachment of rubber hoses. If a hollow-ground knife is used, the contact surface of the water-jacket must be curved to fit it. To attach the water-jacket to the knife, the ends of the contact plate are interposed between the clamp screws and the knife. Sufficient space should be left between the knife edge and the top of the water-jacket to accommodate at least one section ; otherwise it may be difficult to start a ribbon. G. M. F.

Staining Malaria Parasites.—J. W. FIELD (" Further Note on a Method of Staining Malaria Parasites in thick Blood Films," *Trans. Roy. Soc. trop. Med. & Hyg.*, 1941, **35**, 35–42, 3 pls.). The Romanowsky principle is applied to rapid staining with an isotomic aqueous solution of stain. Blood films should be about the size of a shilling and not too thick : the dried thick film should not be so thick that the hands of a watch cannot be seen through it. Films are ready to stain as soon as they cease to be obviously moist. Fixation is unnecessary. Two solutions are used, methylene blue-azure and eosin, both in isotonic solution adjusted to *p*H 6·6. Solution A is composed of methylene blue 0·8 gm., azure i 0·5 gm., disodium hydrogen phosphate (anhydrous) 5·0 gm., potassium dihydrogen phosphate (anhydrous) 6·25 gm., distilled water 500 c.c. Solution B contains eosin 1·0 gm., disodium hydrogen phosphate (anhydrous) 5·0 gm., potassium dihydrogen phosphate (anhydrous) 6·25 gm., distilled water 600 c.c. Films are dipped for 1 second in Solution A ; rinse by waving gently in clean water for a few seconds until stain ceases to flow from the film and the glass of the slide is free from stain. Dip for 1 second in solution B ; rinse by waving gently for 2 or 3 seconds in clean water. Place vertically against a rack to drain and dry. Malarial parasites show blue cytoplasm, chromatin dark purplish-red, pigment unstained yellow of varying shades. Leucocytes show deep blue nuclei, cytoplasm pale blue, granules eosinophilic large dull red, neutrophilic small pale purple. G. M. F.

Clearing Embryos in toto.—C. W. NICHOLS., Jr. (" A Note on the Use of Synthetic Glycerol (Shell) in the Clearing of Embryos *in toto*," *Stain Technol.*, 1941, **16**, 37–8). Recently Williams [*Ind. Eng. Chem.*, 1938, **16**, 630] announced the synthesis of glycerol from petroleum gases. The synthetic glycerol (Shell) has produced no ill effects when tested pharmacologically, and is now shown to be suitable for clearing embryos. Initial clearing of the embryos was done with 5 p.c. KOH, although with smaller specimens only 1–2 p.c. KOH was used, 1–2 days being sufficient. Staining of ossification centres was accomplished with 0·1 p.c. alizarin red S solution in distilled water. The alizarin red was added, drop by drop, with constant stirring, to a 2 p.c. sodium carbonate solution, till a uniform reddish-purple mixture was obtained. The specimens were transferred directly from the KOH to the staining mixture. Complete staining required 12–24 hours. After completion of the staining the embryo was transferred to a 25 p.c. solution of synthetic glycerol (Shell) and allowed to remain from 3–5 days, then into 50 p.c. synthetic glycerol for 3–7 days. Final clearing is carried out in pure synthetic

glycerol. A small crystal of thymol must be added to all glycerol solutions to prevent contamination by fungi. G. M. F.

Phosphotungstic Acid Hæmatoxylin Staining of Formaldehyde-fixed Nerve Tissue.—J. P. MULLEN and J. C. MCCARTER ("A Mordant Preparing Formaldehyde-Fixed Neuraxis Tissue for Phosphotungstic Acid Hæmatoxylin Staining," *Amer. J. Path.*, 1941, **17**, 289–91). Tissues fixed in 4 p.c. aqueous formaldehyde sometimes for several years may be used. Blocks are cut not over 5 mm. in thickness and washed in running water for 6–12 hours and then passed up to 95 p.c. alcohol in the usual manner. Blocks are completely dehydrated with normal butyl alcohol, two changes of 4 hours each. Tissues are embedded in paraffin, sectioned, and taken down to water. Sections are placed in chromium chloride mordant for 2 hours or up to 48 hours, and then rinsed in distilled water and finally in 0·25 p.c. aqueous potassium permanganate solution for 10–15 minutes. Sections are then rinsed in distilled water, bleached till sections have lost the brown tinge in 5 p.c. oxalic acid, again rinsed in distilled water, and stained with Mallory's phosphotungstic acid hæmatoxylin for 6–12 hours, decolorized in 95 p.c. alcohol, two to three changes, dehydrated, cleared and mounted. G. M. F.

Sectioning Hard Tissues.—A. RANDALL and A. W. C. MENZIES ("Histological Sectioning of Hard Tissues by a New Technique," *Science*, 1941, **93**, 189–90). Fluid methacrylates permit sections of hard and brittle materials to be obtained. The resin undergoes polymerization to a solid embedding medium *in situ*. Dry tissues are embedded directly or are dehydrated in alcohol, acetone or dioxan and cleared in xylol. Impregnation is carried out, often at reduced pressure, in monomeric methyl methacrylate in a test tube for 12 hours, changing it three times; 0·05 p.c. benzoyl peroxide is added as a catalyst for polymerization. A portion of the catalyzed monomer is heated for 24 hours at 40° C.; the hardened contents of the test tube are freed by breaking the glass, and sections are ground by petrographic technique. If serial sections are required thin wafers are first cut and affixed to "plexiglass" slides with the partial polymer; polymerization is completed for 6 hours at 40° C. Sections may be stained with safranin and methylene blue. G. M. F.

Mounting Giemsa Preparations.—F. COULSTON ("The Use of Diaphane for Mounting Giemsa Type Preparations," *Amer. J. clin. Path.*, 1941, **26**, 869–73). Smears and tissue sections stained with Giemsa or any other of the Romanowsky stains become decolorized when mounted in balsam. This is due to the formation of acid by oxidation. Diaphane does not become acid, and is therefore far preferable to balsam or cedar oil. G. M. F.

Plastic instead of Coverglasses.—V. SUNTZEFF and I. SMITH ("The Use of Plastic as a Substitute for Coverglasses," *Science*, 1941, **93**, 158–9). Hæmatoxylin and eosin mounted in Canada balsam and mounted under plastic instead of coverglasses faded in 4–5 months. G. M. F.

Staining Tubercle Bacilli.—O. W. RICHARDS ("The Staining of Acid Fast Tubercle Bacteria," *Science*, 1941, **93**, 190). Efforts were made to determine what compound in the tubercle bacilli produces the acid fastness. The only substance obtained from the bacilli that is acid fast was mycolic acid. The degree of fastness, however, varies greatly according to the dye used. While the carbol fuchsin of the Ziehl-Neelsen method stains the acid poorly and is easily removed with acid alcohol, carbol auramine stains it an intense yellow. Mycolic acid is weakly fluorescent to ultra-violet irradiation; auramine causes it to show a bright yellow

fluorescence. The fact that higher counts of tubercle bacilli are obtained by the fluorescence technique may be accounted for by the presence of mycolic acid.

G. M. F.

A Medium for the Detection of Lipolytic Bacteria.—M. P. Starr ("Spirit Blue Agar: A Medium for the Detection of Lipolytic Microorganisms," *Science*, 1941, **93**, 333–4). The medium is made by dissolving 30 gm. of agar, 10 gm. tryptone and 5 gm. yeast extract in 900 c.c. of distilled water by autoclaving; to this is added 25 c.c. of a 20 p.c. cottonseed oil emulsion (10 gm. powdered gum arabic ground thoroughly in 100 c.c. Wesson oil and 400 c.c. warm distilled water) and 50 c.c. of 0·3 p.c. freshly filtered alcoholic spirit blue (National Aniline); bring up to 1 litre with distilled water and autoclave 15 minutes at 15 lb. (121° C.). The medium, which is pale lavender, must be kept in a cool place. Lipolysis is indicated by a permanent deep blue beneath and around the colony. G. M. F.

Romanowsky Stains.—R. D. Lillie ("Romanowsky Staining with Buffered Solutions. III. Extension of the Method to Romanowsky Stains in General," *Stain Technol.*, 1941, **16**, 1–6). Solutions of various Wright, Giemsa, Leishman, and Balch stains (0·3 gm. per 100 c.c.) in equal parts of glycerin and methyl alcohol, and similar eosinates of thiazene dyes give satisfactory wholesale staining of sections without differentiation when buffered with citric acid and sodium phosphate. Previous staining with alum hæmatoxylin adds to the depth, density, and permanence of nuclear staining, but at the same time decreases clarity. A modified Mayer's acid hæmalum has been prepared as follows: 5 gm. hæmatoxylin is dissolved in 700 c.c. of distilled water; sodium iodate 1 gm. and aluminium ammonium sulphate (ammonia alum) 50 gm. are added and dissolved. The solution is allowed to stand over-night, and then 300 c.c. C.P. neutral glycerine and 20 c.c. glacial acetic acid are added. The mixture is ready for immediate use. The reaction of the staining solution varies slightly with the fixing fluid: thus the reaction should be *p*H 4·2 for material fixed in neutral formalin or Orth fixation, *p*H 4·6 for acid formalin, *p*H 5·0 for Zenker formalin, and *p*H 6·5 for ethyl or methyl alcohol or Carnoy fixation. Toluidine blue phloxinate is an excellent stain prepared by precipitating toluidine blue with phloxine in aqueous solution in the proportion of 2 mols. (611·3) of toluidine blue to 1 mol. (829·5) of phloxine B (C.I. No. 778). Since a slight excess of toluidine blue is desirable, 10 gm. toluidine blue in 500 c.c. of distilled water is added to 10 gm. phloxine B in 500 c.c. of distilled water. After standing over-night, the precipitate is collected on hard filter paper with a suction filter and dried. The filtrate should be a very pale clear blue, indicating practical exhaustion of both dyes from the mixed solutions. Clarite and clarite X are superior as mounting media to neutral Canada balsam, but inferior, as regards fading of the preparation, to liquid petrolatum. G. M. F.

Embedding Plant Tissues.—T. E. Rawlins and W. N. Takahashi ("Improved Paraffin Sections for Plant Tissues," *Stain Technol.*, 1941, **16**, 7–8). Two schedules are described: the first causes more distortion but staining is brighter. *Schedule A.* Fix tissues for 48 hours in Karpechenko, Flemming Strong (half strength); wash tissues for 6 hours in running water; place tissues on paraffin oven in a dish filled with the following: 100 c.c. of 10 p.c. glycerin to which has been added 1 c.c. of a 10 p.c. solution of thymol in 95 p.c. alcohol. After all water has evaporated in 10–13 days transfer tissues to normal butyl alcohol and glycerine 1 : 3, for 24 hours; normal butyl alcohol and glycerine 3 : 1, for 24 hours; pure normal butyl alcohol on oven, 24 hours. Transfer tissues to new normal butyl alcohol in a new vial with a new cork to eliminate the film of glycerine present on first

vial and cork. Leave in oven 24 hours. New normal butyl alcohol on oven, two changes, 48 hours in each; agitate vial very gently twice daily while tissues are in butyl alcohol to hasten removal of glycerine. Add new butyl alcohol and place solid blocks of paraffin on a screen suspended in the upper portion of the butyl alcohol. Place the vial in a well-ventilated paraffin oven. Remove screen after 24 hours and allow the remainder of the butyl alcohol to evaporate in the oven. Pour off melted paraffin and add new melted paraffin. Change melted paraffin twice more at 24 hours' interval and imbed after tissues have been in the last melted paraffin for 48 hours. *Schedule B.* After placing tissues in two changes of new normal butyl alcohol on the oven transfer tissues in normal butyl alcohol and cedar oil, 3 : 1 in an open vial and leave till butyl alcohol has evaporated; then transfer to paraffin 25 p.c., 50 p.c., 75 p.c. in cedar oil on the oven, leaving for 24 hours in each case; melted paraffin 24 hours in oven, new melted paraffin 24 hours in oven, new melted paraffin 6 days in oven. Imbed. G. M. F.

Section-Smears.—H. E. WARMKE ("A Section-smear Method for Plant Cytology," *Stain Technol.*, 1941, **16**, 9–12, 3 figs.). Root-tips are placed in Navashin or other fluid containing chromic acid, washed, dehydrated, embedded in paraffin, and sectioned transversely. Sections are stained by De Tomasi's modification of the Feulgen technique [*Stain Technol.*, 1936, **11**, 137], except that the hydrolysis is continued for 45 minutes and a 10-minute washing in running water is introduced between the fuchsin and the first bath in sulphite solution. After dehydrating and mounting in thin Canada balsam, local pressure is applied to the top of the cover-glass with the tip of a scalpel, while the operation is watched under the low power of the microscope. Prolonged hydrolysis softens the tissue and removes sufficient pectic substance so that cells of the section separate readily and may be flattened till the chromosomes lie in a single plane. The slide is permanent. G. M. F.

Clearing Embryonic Material.—H. F. DRURY ("Amyl Acetate as a Clearing Agent for Embryonic Material," *Stain Technol.*, 1941, **16**, 21–2). Amyl acetate is soluble in 95 p.c. alcohol and causes no hardening in objects exposed to its action for prolonged periods. It is useful as a general clearing agent and is specially recommended for refractory material. The following method is satisfactory for whole frog embryos and young tadpoles: 45 minutes to 1 hour in 95 p.c. alcohol, 24 hours in amyl acetate, rinse in toluene, 15 minutes each in three changes of paraffin, imbed. Material so treated may be sectioned at 5μ with comparative ease. G. M. F.

Staining Bacteria and Yeasts.—W. E. MANEVAL ("Staining Bacteria and Yeasts with Acid Dyes," *Stain Technol.*, 1941, **16**, 13–19). The advantage of using acid dyes is that there is better differentiation and less tendency for slime and debris to take the dye. The following dyes were used: acid fuchsin, aniline blue (water soluble), erythrosin, fast acid blue R, fast green FCF, light green SF, yellowish, orseilline, phloxine, and rose bengal. The formulæ used were as follows: *Solution A:* Phenol 5 p.c. aqueous 30 c.c., glacial acetic acid 20 p.c. aqueous 8–10 c.c., ferric chloride 30 p.c. aqueous 4 c.c. To this a 1 p.c. aqueous solution of any of the following dyes: acid fuchsin 1–2 c.c., aniline blue (water soluble) 2–8 c.c., fast green FCF 2–8 c.c., light green 2–8 c.c., fast acid blue R 15 c.c., omitting ferric chloride, orseilline BB, 8 c.c., omitting ferric chloride. *Solution B:* Phenol 5 p.c. aqueous 100 c.c., to which is added 1 gm. of rose bengal, erythrosin, or phloxine, previously dissolved in about 5 c.c. of distilled water. Enough aqueous acetic acid (6–8 drops) are added to cause a trace of cloudiness. Finally add

20–50 c.c. of 95 p.c. alcohol. These dyes may be used for counter-staining after acid fast or Gram staining. G. M. F.

Differentiating Bone and Cartilage.—T. W. Williams ("Alizarin Red S and Toluidine Blue for Differentiating Adult or Embryonic Bone and Cartilage," *Stain Technol.*, 1941, **16**, 23–5). Specimens are fixed in 10 p.c. formalin, stained for 1 week in a solution of 0·25 gm. of toluidine blue in 100 c.c. of 70 p.c. alcohol, macerated for 5–7 days in a 2 p.c. solution of potassium hydroxide, counter-stained for 24 hours in a 0·001 p.c. solution of alizarin red S in 2 p.c. aqueous potassium hydroxide, dehydrated in cellosolve, and cleared in methyl salicylate. The method can be used for staining, *in toto*, the bones and cartilages of mature specimens of *Urodela* as well as the developing bone and cartilage of the embryonic human, cat, pig, and rat. In the adult and embryonic forms thus treated the soft tissues are cleared while the osseous tissues stain red, the cartilage blue. G. M. F.

Permanent Mounts of Whole Decolorized Leaves.—J. C. Bates ("A Method for Making Permanent Mounts of Portions of Decolorized whole Leaves," *Stain Technol.*, 1941, **16**, 38). Decolorizing, dehydrating, and clearing can be carried out in a wide-mouthed bottle. After decolorizing the leaves are dehydrated with ethyl alcohol, the dehydration being completed in carbol-xylol, made by adding 1 part of phenol crystals to 3 parts of xylol. The leaves are then cleared by treating with two changes of pure xylol. After clearing the leaves are removed from the bottle one at a time and placed in a shallow dish of xyline. Small portions of the leaf, from 5–10 mm. square, are cut out with scissors, mounted on a clean slide in a drop of hydrax, and covered with a cover glass. When these preparations are examined under the microscope, the different layers of leaf tissue may be observed by simply raising and lowering the objective. G. M. F.

The Reaction of Stains and Bacteria.—T. M. McCalla ("The Reaction of Certain Stains with Bacteria," *Stain Technol.*, 1941, **16**, 27–32). Evidence is brought forward to show that the reaction of stains with bacteria is an adsorption exchange process, reaching stoichrometrical proportions. By using bacteria saturated with Mg^{++} or H^{+} at specific concentrations it was demonstrated that when a stain acts on a bacterial cell it replaces some ion already adsorbed by the cell. Basic stains act as cations, replacing similarly charged ions from the bacterial system. Basic stains decrease while acid stains increase the *p*H value of a suspension of H-bacteria, that is to say, bacteria treated with H^{+}. From the increase in acidity obtained by displacing H^{+} with a basic stain there appears to be a maximum exchange value. Stains apparently react with the bacterial cell at the same positions as do inorganic ions. G. M. F.

Wax Light Filters.—E. D. Crabb ("A Multicoloured Wax Light Filter and Dissecting Chamber for Microscopes," *Rev. Sci. Instruments*, 1941, **12**, 3). A simple filter with four colours may be made by compressing four triangular bits of previously coloured, translucent, plastic wax between a watch crystal and glass plate. Balls of stock wax were prepared by colouring "flexo wax C" with glycolours (Glyco Products Co., 230 King St., Brooklyn, New York). By kneading together bits of differently coloured stock waxes a variety of shades may be produced. Wax filters may be used with dissecting or oil-immersion microscopes. G. M. F.

A Resin Paraffin Mixture.—R. A. Groat ("New Paraffin Resin Infiltrating and Imbedding Media for Microtechnique," *Science*, 1941, **93**, 311–12). Paraffin, melting-point 56°-58° C., mixes readily with a water-clear, LX–291, hydrocarbon

resin produced by the Neville Co., Pittsburgh, Pa. By changing the proportions of resin and paraffin the mixtures differ in hardness at a given temperature, but not essentially in melting range. As the resin content is increased so is the hardness. Mixtures, containing 5, 10, 20, and 30 p.c. of resin, show a melting range of several degrees, with a minimum about 55° C. An average oven temperature of 59° C. is satisfactory for all. Weighed portions of paraffin and resin are mixed at 170° C. and filtered; tissues are infiltrated in pure paraffin or a 5–10 p.c. mixture, embedded in a harder medium. For comparison a 10 p.c. medium allows sections to be cut of from 4 to 12μ; a 50 p.c. medium permits only 1μ sections. G. M. F.

A Substitute for Cover-Glasses.—S. F. PENNY ("A Substitute for Cover Glasses in Mounting Pathological Sections," *Canad. J. med. Technol.*, 1940, **2**, [No. 2. March]). Due to rise in price and lack of German supply the author tried various thicknesses of cellophane, plastecele, and mica, using gum dammar as mounting medium. All proved unsatisfactory. Attempts were made to find a transparent plastic material which could be applied in liquid form, drying with a hard, smooth surface. Clear Duco was fairly satisfactory, but a new quick-drying clear lacquer has proved the best material so far. The lacquer used is C.I.L. Special Slide Lacquer XB 6965–(S.R. 2452: I.R. 23–710). Retail price, January, 1940, was $7.50 per gallon, obtained from Paint and Varnish Division, Canadian Industries, Ltd. This lacquer has an advantage over Duco in that it is soluble in xylol, and may be applied to sections direct after removal from xylol. Apply the lacquer by brushing it lightly over the section and the surrounding slide with a camel-hair brush about 1·5 cm. in width. The sections are then allowed to dry at room temperature and after 15 minutes are ready to be examined. The lacquer surface smoothes out and brush marks disappear. It has remained clear after exposure to light for several months. Under high-power oil immersion the cell detail is slightly hazy. The mixture index was estimated to be 1·4835, and this is reputed to be the nearest index to glass of any of the quick-drying lacquers. If a high-power dry objective with compensating collar is used, the refractive error can be corrected and cell detail becomes quite clear. S. G. L.

Hydracarina.

Benthic Fauna of a Danish Lake.—BERG ("Studies on the Bottom Animals of Esrom Lake," *K. danske vidensk. selsk. Skr.*, 1938 (9), **8**, 1–255 (Hydrach, pp. 120–30), figs. 124–35). With one or two exceptions most of the species now listed have already been recorded for the Danish fauna. The author records that the benthic distribution maxima appear to be in the 5m and in the 14m zones, with the minimum about the 8m level. The sublittoral zone appears to be preferred to the naked shingle of the littoral zone, but where this zone has a cover of vegetation which will protect the shallow water from agitation, the Hydracarina will resort to it. A list is given of certain species known to hibernate as imagines.

BM/HNDH

Watermite Eggs in Salamander Spawn.—INUO ("Some Observations on a Watermite found on the Eggmass of an Amphibian," *Annot. zool. Jap.*, 1938, **17**, 537–40, 3 text-figs.). Though only published in 1938, note is stated to have been written in 1934. Material was identified by Uchida as *Piona variabilis* var. *dispersa*, and was taken from the spawn of *Hynobius nigrescens* in a pool about 5500 feet above sea level, where *Hynobius* is stated to be abundant. Author has not hitherto appeared as a worker among watermites, and when he writes of teleiophans of *Piona* swimming after being dislodged from the spawn of *Hynobius*,

one is inclined to think that it is the nymphal stage prior to the teleiophan that is meant. The tenor of the note brings to mind Halbert's suggestion made some years ago of the possibility of the existence in some species of watermites of a second nymphal stage, to which a more critical study in this case might have given some support. BM/HNDH

A Limnesiad Teleiophan.—MEUCHE ("Zum Teleiophanstadium der Wassermilbe *Limnesia maculata* (O. F. Müller)," *Zool. Anz.*, 1936, **116**, 264–7, 6 text-figs.). Author describes the teleiophan of *Limnesia maculata* which he found in a colony of the blue-green alga *Glœotrichia intermedia.* Colour ranges from greyish-green or brown to bright red, while the skin is covered with sharp-pointed papillæ. A small area of unknown function is suggested by Viets to author to represent the maxillæ, and it is proposed by Meuche to name it the maxillary area.
BM/HNDH

Unusual Habitat for Watermites.—VIETS ("Eine neue, die erste Süsswassermilbe (Hydrachnellæ, Acari) aus tropischen Pflanzengewässern," *Zool. Anz.*, 1939, **128**, 69–77, 10 text-figs.). Dr. Geijskes found in the leaf axils of certain epiphytic plants (*Ananas*) growing in Surinam 6 males, 18 females, and 1 nymph of a species of Arrenurus which differed in some respects from the recognized Arrenurid subgenera. In these leaf axils water had accumulated so as to form small aquaria, wherein besides watermites certain species of insects, which pass part of their existence in water, had taken up their abode. As these watermites could only reach such places during the transition stage between larva and nymph through the agency of aerial insects, Viets regards these mites as having acquired a more or less established habitat, nutriment being obtained at the expense of the other minute creatures also inhabiting these "aquaria." This new Arrenurid in some respects approaches subgen. *Micruracarus*, but the projecting tongue on the fourth segment of the palp which is opposed to the claw-like fifth segment is entirely wanting in the new species. A curious feature is also the fact that when the mites were abstracted by pipette from the water, they adhered to the pipette, though no suctorial glands could be detected. Viets suggests that some secretion may be liberated. The new species is *Arrenurus (Micruracaropsis) phytotelmaticola* n.subg. n.sp. BM/HNDH

Watermites from Oceanic Islands.—VIETS ("Süsswassermilben (Hydrachnellæ, Acari) von Ozeanischen und Pseudo-ozeanischen Inseln," *Zool. Anz.*, 1939, **128**, 206–8). Author summarizes our present-day knowledge of work done in the Bismarck Archipelago, New Caledonia, Hawaii, Fernando Po, and the Azores since 1887. BM/HNDH

Romanian Hydracarina.—HUSIATINSCHI ("Hydracarinen aus der Dobrogea (Rumänien)," *Zool. Anz.*, 1939, **127**, 102–6, 9 text-figs.). Collections made in the Dobrogea by Dr. A. Rosca are recorded. Among these *Eylais szalayi* is a new species, while author treats *Eylais degenerata hispanica* of Viets as a synonym of *Eylais* (*Proteylais*) *variabilis* Thor. BM/HNDH

Brazilian Watermites.—VIETS ("Brasilianische Wassermilben. V," *Zool. Anz.*, 1938, **121**, 21–4, 4 text-figs.). This is a continuation of an earlier series of preliminary descriptions of new species and subspecies. Two new Arrenuri are remarkable, *A. mirabilis* in respect of the globate form of the appendage, with its broad terminal hooks, and *A. cultriger* in that the bristle equipment of the second segment of the palpi is unusual. BM/HNDH

New Uruguayan Hydracarina.—VIETS (" Über Hydrachnellæ aus Uruguay," *Zool. Anz.*, 1938, **121**, 131–6, 5 text-figs.). Two new species are recorded from Uruguay. *Limnesiopsides* is a new subgenus of *Limnesia* created to accommodate *L.* (*L*) *pectungulatus*, n.sp. This has the genital plates so placed as to suggest that the specimen is a female. The presence of a penial organ shows it to be a male. *A. curticaudatus* is stated by Viets to resemble *A. birgei* Mars, and, as its name suggests, possesses a short appendage. In view of Münchberg's work on variation between mature and immature Arrenurid female imagines, it would have been more satisfactory if it had been possible to state that ample material was obtained to justify assumption of maturity. Certain Limnesiad species designated by Marshall in 1927 and 1932 are treated by Viets as *Limnesia anomala*, var. *marshallæ*, n. var. BM/HNDH

Halacarids from France.—ANDRÉ (" Halacariens récoltés dans le Bassin d'Arcachon en Septembre, 1938," *Bull. Mus. Hist. nat. Paris.* (2) 1939, **11**, 118–22, text-fig.). After discussing the halacarids to be found along the Channel and Atlantic coasts of France, and the varying depths therefrom, the author concludes with a note of captures made by him in the Arcachon basin between Cap Ferret and Pyla-sur-Mer. BM/HNDH

Belgian Spelean Hydracarina.—LERUTH (" La Faune de la nappe phréatique du gravier de la Meuse à Hermalle-sous-Argenteau," *Bull. Mus. Hist. nat. Belg.*, 1938, **14**, No. 41. " La Biologie du domaine souterrain et la faune cavernicole de la Belgique," *Mém. Mus. Hist. nat. Belg.*, 1939, **87**, 1–506, text-figs. and pls.). The hydracarina collected are summarized in the larger work (pp. 413–6, fig. 51), while on page 68 the definition of " nappe phreatique " is given in the following words : " Le nom de nappe phreatique a été donné à l'ensemble des eaux souterraines de toutes les formations géologiques, que nous pouvons atteindre par des puits (*phreatos*) d'ou les expressions de ' domaine phréatique ' et de ' Faune phréatique ' pour désigner ce milieu biologique et sa population." The work as a whole is a valuable contribution to a subject which has received comparatively little attention in this country. BM/HNDH

Watermites as Turtle Food.—MARSHALL (" On the Occurrence of Water Mites in the Food of Turtles," *Amer. Midland Nat.*, 1940, **24**, 361–4, 4 text-figs.). Mr. K. F. Lagler examined the contents of the digestive organs of 965 turtles belonging to seven species. Only two species, however, were found to have swallowed watermites, viz. the Western Painted Turtle (*Chrysemys picta marginata*) and the Snapping Turtle (*Chelydra serpentina*), and as only 50 out of 965 yielded mites, the author concluded that they cannot be considered as of any use as turtle food. The digestive juices of these turtles had little effect on the mites even when the mites were recovered near the end of the digestive tract. This is in contrast to the author's observations on the mites recovered from the digestive tract of fishes where much disintegration had taken place. One of the turtles yielded the hitherto unknown female of *Limnesia* (*Tetralimnesia*) *wawaseea* Mar. The communication also describes the previously unknown female of *Hygrobates multiporus* Koen. In this case the female was taken from the stomach of the Blue Heron (*Ardea herodias*). BM/HNDH

Protozoa.

Eocene of Alabama.—J. A. CUSHMAN and J. B. GARRETT (" Eocene Foraminifera of Wilcox Age from Woods Bluff, Alabama," *Cont. Cush. Lab. For. Res.*, 1939, **15**, No. 211, 77–89, pls. 13–15). This may be regarded as supplementing the

paper by Cushman and Ponton "An Eocene Foraminiferal Fauna of Wilcox Age from Alabama," *Cont. Cush. Lab. For. Res.*, 1932, **8**, pt. 3. Many of the species are identical, and some have affinities with species described by Schwager from the Eocene of Libya and Egypt. Five new species are recorded: *Darbyella wilcoxensis, Uvigerina wilcoxensis, U. alabamensis, Asterigerina wilcoxensis, and A. alabamensis.* The two new species of *Uvigerina* are almost the earliest records for the genus. They appear to be primitive forms intermediate between *Bulimina* and *Uvigerina*, but having the apertural characters of the latter. An interesting record is that of *Spirillina selseyensis* Heron-Allen and Earland, 1909, previously known only from the Eocene of Selsey Bill and Biarritz. The plates are good. A. E.

Submarine Cores with Eocene Foraminifera.—J. A. CUSHMAN ("Eocene Foraminifera from Submarine Cores off the Eastern Coast of North America," *Cont. Cush. Lab. For. Res.*, 1939, **15**, 49–76, pl. 9, figs., 9–34 and pls. 10–12). Two cores taken by the "Atlantis" off the Atlantic coast of North America—roughly to S.E. of New York—were found to have penetrated sediments of Eocene Age. The cores—Nos. 12–36, taken in 880 metres (39° 50′ N., 70° 58′ W.) and Nos. 21–38 taken in 1565 metres (38° 58′ N., 72° 28′ 30″ W.)—evidently represent different conditions, although both contain *Hantkenina alabamensis* C., a typical Upper Eocene species. The two faunas are somewhat alike, Core 12–36 being much richer in species of foraminifera than Core 21–38, which on the other hand is very rich in Radiolaria. In Core 12–36 Radiolaria are very rare. The fauna is very interesting in its relationships with other known Eocene material, as the locality is far removed from any of the shore outcrops in Cuba, Venezuela, and Mexico. Fifteen new species and two new varieties are described and excellently figured. A. E.

A New Elphidium.—W. A. MACFADYEN ("*Elphidium icenorum:* A New Species of Foraminifera from the Sub-Recent Deposits of the Cambridgeshire Fenland," *Ann. Mag. Nat. Hist.*, 1939, Ser. 11, **4**, 610–13, pl. 15). About 70 samples of silts and clays from the post-glacial (often brackish water) deposits of the English Fenlands, and about 140 indigenous species of Foraminifera have been identified. They are all familiar living forms, with the single exception of *Elphidium icenorum*, which was found in only four of the samples. This is a small but very characteristic species, in which the retral processes give an effect of herring-bone ornament to the septal areas which is very distinct from any other species of the genus. It is interesting also in respect of the fact that the deposits in which it has been found can be dated between, say, 2500 B.C. and A.D. 1000. Whether the species is still living is unknown. As its habitat appears to have been one of estuarine or brackish water, it may possibly be rediscovered under similar conditions to-day, but not in the open sea. A. E.

Pliocene of Kar Nicobar.—J. A. CUSHMAN ("Notes on some Foraminifera described by Schwager from the Pliocene of Kar Nicobar," *Trans. Palæont. Soc. Japan*, No. 81. *Jour. Geol. Soc. Japan*, 1939, **46**, No. 546, 149–54, pl. 10 (6)). The examination of material from Schwager's original locality has made it possible to interpret some of his species more accurately than his figures and descriptions permitted. Thirteen of Schwager's species are described and refigured, many being transferred to other genera. *Nodosaria fistuca*, *N. lepidula* and *N. grandigena*, are now referred to the genus *Ellipsonodosaria*; *Dimorphina striata* becomes *Siphogenerina striata* (Schwager) and *Fissurina staphyllearia* is referred to *Entosolenia*, which many workers refuse to accept as a genus. *Discorbina sacharina* must be regarded as a mere variety of *Globorotalia menardii* (d'Orbigny) and *Anomalina cicatricosa* should be referred to the genus *Cibicides*. A. E.

New Species from Cuba.—PEDRO J. BERMUDEZ and JOSE T. ACOSTA ("Resultados de la primera expedicion en las Antillas del Ketch Atlantis bajo los auspicios de las Universidades de Harvard y Habana.—Nuevas Especias de Foraminiferos Recientes," *Mem. Soc. Cub. Hist. Nat.*, 1940, **14**, No. 1, 55–8, pl. 9). Three new species and a new variety are described and figured: *Cystammina aguayoi* is very distinctive. Though akin to *C. pauciloculata* (Brady) it differs in its globose form and in the selective constructions of its tests, which are built up of sponge spicules imbedded in the ferruginous cement usually employed by this genus. *Chrysalogonium brodermanni* is particularly interesting because the genus is practically unknown in recent deposits, though represented by various fossil species. It occurs only in a single dredging from 810 fathoms, where it is frequent. *Cassidulina palmeræ* belongs to the *subglobosa* group of the genus, and in its coarsely reticulate ornament is comparable with *C. decorata* Sidebottom from the Pacific. So far as the Abstractor is aware no species of *Cassidulina* with surface ornament has hitherto been recorded except from the Pacific. The new variety *Gaudryina quadrangularis* Bagg, var. *antillana* appears to be merely a small form of the type species.
A. E.

Foraminifera Up to Date.—J. A. CUSHMAN ("Foraminifera, Their Classification and Economic Use," Harvard University Press, 1940, 3rd edn., 535 pp., 79 pls., text-figs. $6.00). So much work has been published since the second edition of this indispensible work in 1933 that a new edition will be generally welcomed. Its usefulness is increased by the fact that the new edition not only reproduces the second edition, together with descriptions of more than 100 subsequently erected genera, but also includes the "Illustrated Key to the Genera" which was originally issued in 1933 as a separate publication. The family groups remain unchanged except for certain modifications in the Orbitoids. A few genera have been declared invalid and revised under older names not previously recognized. The discussions of the various families are useful, several genera being transferred to other families, and the geological range of other genera is widely extended. As previously but little space is devoted to the biological study of the Foraminifera, and the recent work of Dr. Earl H. Myers on *Spirillina* and *Patellina* is overlooked. It is impossible within the short limits of an abstract to call attention to many points of the greatest interest, or to the omission of others which would have increased the value of the work. It must suffice to say that this new edition is indispensable to every student of the Foraminifera, and that Dr. Cushman is to be congratulated on his latest attempt to make the group more accessible both to the amateur and to the professional student.
A. E.

Recent Species of Bulimina.—J. A. CUSHAMN and F. L. PARKER ("The Species of the Genus *Bulimina* having Recent Types," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 215, 7–23, pls. 2, 3). Supplementing an earlier paper on the species of *Bulimina* named by d'Orbigny in 1826 (*Cont. Cush. Lab. For. Res.*, 1938, **14**, 90) the authors now discuss the remaining Recent species, using topotype material, or reproducing the original figures and descriptions. There is a supplementary list of species ascribed to *Bulimina* which should now be transferred to other genera. A new sub-genus *Desinobulimina* is proposed for those species in which the aperture of the last formed chambers becomes terminal, connecting with the earlier apertures by an internal trough which is joined to one side of the aperture towards the front, and projects above it at the back in the form of a tooth—type *B. auriculata* Bailey, 1851.
A. E.

The Genus Stensioina.—J. A. CUSHMAN and A. L. DORSEY ("The Genus *Stensioina* and its Species," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 214, 1–6,

pl. 1). The genus *Stensioina* was erected by Brotzen in 1936 for the well-known cretaceous fossil *Rotalia exsculpta* Reuss, 1860. While possessing some characters resembling *Anomalina* or *Cibicides*, the aperture is similar to that of *Gyroidina*, and the genus should be included in the Rotaliidæ. It appear to be restricted to the Upper Cretaceous of Europe and America. Five species are described and figured, two of them being new, viz. *S. labyrinthica* and *S. americana*. A. E.

Pacific Textulariidæ.—C. G. LALICKER and IRENE MCCULLOCH ("Some Textulariidæ of the Pacific Ocean," *Allan Hancock Pacific Expeditions*, 1940, **6**, No. 2, 115–43, pls. 13–16). Some interesting facts concerning the distribution and migration of species have come to light in the course of study of material collected on the Eastern Pacific coasts of America. Several of d'Orbigny's species of *Textularia* originally described from the West Indies are rather common in the collections. They may have migrated before the uplifting of the land bridge between North and South America in Pliocene times, or during one of the warm inter-glacial stages in Pleistocene times when the sea level was higher than at present. *Textularia articulata* d'Orbigny reported as living off the West coast of France, and common in the Miocene of the Vienna Basin, France, Florida, and New Zealand is found living off the West Coast of America from California to Peru. *T. lythostrata* (Schwager) known from the Pliocene of Kar Nicobar and New Guinea has also been found in the collections. Results so far indicate that the majority of new species is to be expected in the Gulf of California and in the vicinity of the Galapagos Islands. New species are *Textularia astutia*, *T. aura*, *T. lancea*, *T. lauta*, *T. orbica*, *T. plaga*, *T. ramosa*, *T. secasensis*, *T. scrupula*, and *T. vola*. The plates are good. A. E.

Irish Boulder Clays.—W. A. MACFADYEN ("Foraminifera in Boulder Clays from the Wexford Coast of Ireland (With a Note on the Generic Name *Streblus* Fischer)," *Geol. Mag.*, 1940, **77**, No. 4, 276–82, 2 text-figs.). The Upper and Lower Boulder Clays are sharply separated at Clones, Co. Wexford, and have some physical differences which were supposed to represent a considerable difference in age. The samples were too small for convincing evidence, but so far as investigation has gone there seems little hope of distinguishing the two clays by their micro-fossils. The common species occur in both, as also do the same derived Cretaceous Foraminifera. The rare species, one or two specimens only being found in a sample, are unsatisfactory; they are easily overlooked and larger samples would probably indicate that they were not confined to one or other of the clays. The evidence shows, however: (1) That the climate was colder than that of the present Irish coast; (2) That the recorded Foraminifera are purely marine and not brackish water forms; (3) That the derived chalk fossils are all minute species easily transported either directly from an outcrop of the chalk or from some other deposit containing derived fossils. The lists of species obtained are not dissimilar from those in Joseph Wright's "Post-Tertiary Foraminifera of the North-East of Ireland," when allowances are made for changes in nomenclature, the principal difference being that *Nonion orbicularis* (Brady) is common in the Clones material but absent from the lists of Wright, who records *N. depressulus* (Walker and Jacob) in quantity. Measurement of a number of specimens of both species from various localities, including some of Wright's specimens, leads the author to the conclusions that *N. orbicularis* should be regarded as a variety of *N. depressulus*, the points of difference being due to developments under colder conditions, and that some of Wright's specimens of *N. depressulus* must be regarded as *N. orbicularis*. The genotype of *Rotalia* Lamarck is *R. trochidiformis* Lamarck, and Colonel L. M. Davies has shown that it is probably not congeneric with the common species

Rotalia beccarii (Linné). The author suggests the revival of the generic name *Streblus* Fischer, 1817, for *Rotalia beccarii* and associated species. A. E.

Eocene of Alabama.—J. A. CUSHMAN (" Midway Foraminifera from Alabama," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 222, 51–73, pls. 9–12). Midway material (Lowest Eocene) collected in Alabama contains numerous Foraminifera, two samples in particular having quite different faunas and are of special interest in showing the same relationships in nearly all the species as have already been recorded by Mrs. H. J. Plummer in her work on the " Midway of Texas " (1927). Her material has been available for comparison in the preparation of this paper. Ten new species are described and figured, belonging to the genera *Ammobaculites, Dorothia, Listerella, Dentalina, Frondicularia, Gumbelina, Rectogumbelina, Eouvigerina, Ellipsonodosaria,* and *Discorbis.* A. E.

New Buliminæ.—J. A. CUSHMAN and F. L. PARKER (" New Species of *Bulimina*," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 221, 44–8, pl. 8, figs. 12–20). Figures and describes two new species *Bulimina aspera* and *B. pectinata,* also a new variety *B. kickapoensis* var. *pingua,* all from various Upper Cretaceous beds in Texas. Also *B. eccentrica* and *B. versa* from Eocene of France. *Tritaxia minuta* Marsson, 1878, which is a *Bulimina*, is renamed *B. marssoni,* the specific name being preoccupied by Hantken, 1883 ; and *B. baccata* Fornasini, 1901, is transferred to its synonym *B. gibba* Fornasini, 1901, the first specific name having been preoccupied by Yokoyama in 1890. A. E.

Lower Oligocene of Alabama.—J. A. CUSHMAN and W. MCGLAMERY (" New Species of Foraminifera from the Lower Oligocene of Alabama," *Cont. Cush. Lab. For. Res.*, 1939, **15**, 45–9, pl. 9, figs. 1–8). A preliminary note describing new species. *Dentalina* (1), *Globulina* (1), *Nonion* (1), *Nonionella* (1), *Angulogerina* (1), *Virgulina* (1), *Discorbis* (1). A. E.

New Species.—J. A. CUSHMAN and D. L. FRIZZELL. (" Two New Species of Foraminifera from the Oligocene, Lincoln Formation, of Washington," *Cont. Cush. Lab. For Res.*, 1940, **16**, No. 220, 42–3, pl. 8, figs. 10–11). Describes and figures *Eponides kleinpelli* and *Cassidulina galvinensis* pending publication of a paper on the fauna of the Lincoln formation. A. E.

Cretaceous Anomalinidæ.—J. A. CUSHMAN (" American Upper Cretaceous Foraminifera of the Family Anomalinidæ," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 218, 27–40, pls. 5–7). Pending the publication of a large report on the Foraminifera of the American Upper Cretaceous, the author describes and refigures a large number of species of the genera *Anomalina, Planulina,* and *Cibicides* which have proved to be good index fossils for stratigraphic purposes. There are no new forms. A. E.

The Genus Candorbulina.—J. A. CUSHMAN and A. L. DORSEY (" Some Notes on the Genus *Candorbulina*," *Cont. Cush. Lab. For. Res.*, 1940, **16**, No. 219, 40–2, pl. 8, figs. 1–9). The genus was established in 1933 by Jedlitschka for a single species *C. universa* from the Miocene of Czechoslovakia, and has not since been recorded in literature. It appears to be confined to the Miocene, and being pelagic would have a wide distribution. The author has specimens from Hungary, Austria, Egypt, and several American localities. It is described as having a text similar to *Orbulina*, but with the apertures consisting of one or more rings of small openings coinciding with the edge of contact of the earlier attached *Globigerina* chambers with the final adult sphere. It is suggested that *Candorbulina* originated from *Globigerinoides* and gave rise to *Orbulina,* itself becoming extinct. The figures are

not very good and give the impression that the genus merely represents abnormal specimens of *Orbulina universa* d'Orbigny. A. E.

Natural History of Protozoa.—R. Hegner ("La historia natural de los protozoarios parasitós," *Rev. Soc. Mexic. Hist. Nat.*, 1939, **1**, 19–27). This is a general and popular review of the ecology, especially the host-parasite relationships, of the protozoa parasitic in man. C. A. H.

Osmiophilic Structures in Protozoa.—R. F. MacLennan ("A Quantitative Study of Osmic Acid Impregnation in Protozoa," *Trans. Amer. Micr. Soc.*, 1940, **59**, 149–59, 2 figs.). It is generally accepted that consistency of impregnation with osmic acid affords a criterion for the differentiation of true Golgi bodies from other granular inclusions. This assumption has been critically examined in the present paper, using protozoa (*Actinosphærium*, *Epidinium*, *Eudiplodinium*, *Haptophrya*, *Ichthyophthirius*, *Metadinium*, and *Ostracodinium*) as material. The percentage of impregnation was studied for four types of osmiophilic granules (excretory, digestive, ectoplasmic, and fatty acid), all of which can be identified—independently of impregnation—by size, shape, or localization. It was found that whenever these granules are present 100 p.c. are impregnated. Apparent differences in the impregnation of these types of granules are due to cyclical changes in the aggregation of these granules or to their absence, due to metabolic changes associated with different phases of the life cycle of the organisms. These granules cannot be separated on the basis of the osmic acid reaction alone, therefore none can be identified with true Golgi bodies to the exclusion of the others; but as long as the osmic methods are accepted as the chief criterion for the comparison of granules in protozoa with the Golgi apparatus in metazoan cells, all the various types of osmiophilic granules have to be accepted as representing true Golgi bodies. C. A. H.

Races in Giardia.—P. C. Waters, A. R. Fiene, and E. R. Becker ("Strains in *Giardia ondatræ* Travis, 1939," *Trans. Amer. Micr. Soc.*, 1940, **59**, 160–2). A comparison of two strains of the intestinal flagellate of the musk-rat, *Giardia ondatræ*. In each strain measurements were made of 100 individuals as follows: body breadth at the level of the centres of the nuclei; breadth across ends of lateral shields (sucking discs); anterior end to level of centres of nuclei; level of centres of nuclei to level of ends of lateral shields; level of ends of lateral shields to posterior end; and total length. The two strains were found to differ significantly in respect to every measurement made, though their contours were practically identical. C. A. H.

Body-volume of Infusoria.—T. T. Iida ("Cell Volume of *Paramecium*," *Japan. J. Zool.*, 1940, **8**, 395–7, 14 figs.). A comparison of the volume of the body of *Paramecium caudatum* in M/50 and M/1000 balanced salt solutions showed no appreciable difference. Measurements of the ciliates were made from photomicrographic records projected on paper, the ciliates being previously embedded in a viscous fluid containing 0·5 p.c. tragacantha. Calculations of volume were made from the following formulæ: $V_1 = \theta \, lb.^2$ and $V_2 = \psi A^2/1$, where V is the volume, 1 = length, b = maximum breadth of body, A = lateral projection area of the animal, while θ and ψ are form factors. The latter were determined from plasticine models. C. A. H.

Relation of Volume to Weight in Infusoria.—T. T. Iida ("Cell Volume vs. Cell Dry Weight Relation in *Paramecium*," *Japan. J. Zool.*, 1940, **8**, 399–406, 4 figs.). In order to determine the relation of volume to dry weight of the body in

Paramecium caudatum the author used four methods, in two of which the data are obtained from formulæ, in one by "conductance titration," and in another by electrical resistance. These methods are described in detail. The average value of the ratio of dry weight to volume obtained by the four methods was found to be 0·22 gm./c.c. Hence 1 c.c. of Paramecium cell yields on an average 0·22 gm. dry substance. Since 1 c.c. of the ciliate weighs 1·057 gm., 1 gm. of the ciliate contains 0·21 gm. dry substance and 0·79 gm. water. C. A. H.

Conduction of Electricity in Infusoria.—T. T. IIDA ("Electrical conductivity of cytolyzed *Paramecium*," *Japan. J. Zool.*, 1940, **8**, 407–14, 7 charts). For the determination of the electrical conductivity hay cultures of *Paramecium caudatum* were cytolyzed as follows: the ciliates were first acclimatized to a salt solution, then condensed with silk gauze and centrifuged; the supernatant fluid was poured off and 2·4 M/10,000 $NaHCO_3$ was added, mixed and again centrifuged. The whole procedure was repeated three times, using distilled water instead of the carbonate, then 1 p.c. solution of saponin was added and mixed thoroughly. After 30 seconds 1·5 c.c. distilled water was added and the whole preparation stirred vigorously. After 2 minutes' standing the ciliates underwent cytolysis. The preparation was then transferred to the conductivity cell. It was found that the electrical conductivity of the cytolyzed organisms increases automatically with the progress of time, owing to the release of ions. The temperature coefficient is of the order of usual chemical reactions. By exposure to high temperature the conductivity is irreversibly suppressed, showing that some thermolabile substance is involved in the process. This substance was found to be localized in the fine granules suspended in the preparation. It does not induce conductivity change, but this is produced by a second element which is thermo-resistant. C. A. H.

Protozoological Technique.—D. H. WENRICH ("The Morphology of some Protozoan Parasites in Relation to Microtechnique," *J. Parasitol.*, 1941, **27**, 1–27, 4 pls.). The author discusses the relative merits of various methods of fixation and staining of parasitic protozoa, especially of intestinal forms. After comments on the technical processes used, their effect on the protozoa are considered. Experiments have shown that fixation with the usual reagents for shorter periods of time give results as good as longer times. Schaudinn's fluid, acetic acid, alcohol, etc., in weaker solutions were found to fix better than stronger solutions. As regards the organisms themselves, intestinal protozoa may vary in appearance though treated by the same technique; the same applies to different individuals on the same slide. On the other hand, different fixatives and stains may produce different appearances in the same species. C. A. H.

Intestinal Protozoa in U.S.A.—(1) L. V. REARDON ("Incidence of *Endamœba histolytica* and Intestinal Nematodes in a Georgia State Institution," *J. Parasitol.*, 1941, **27**, 89–90); (2) R. M. STABLER ("Intestinal Protozoa in 106 Parasitology Students," *ibid*, 90). The first paper records the results of examination of the inmates of a State institution for *Entamœba histolytica*. The incidence was found to be very high, 40 and 44 p.c. in two groups of 72 and 70 respectively, all the cases being healthy carriers. Among the students (Pennsylvania) reported in the second paper, 34 were positive for protozoa, the parasites being distributed as follows (p.c.): *Entamœba histolytica* 8, *E. coli* 8, *Endolimax nana* 13, *Dientamœba fragilis* 3, *Giardia intestinalis* 11. C. A. H.

Human Leishmaniasis in China.—S. YOUNG ("Kala-azar in Pi-Hsien District, Kiangsu Province, China." II. Findings in Films of Spleen and Liver Puncture Juice and some other Observations in Kala-azar," *J. Shanghai Sci. Inst.*,

1939 (IV), **4**, 265–72, 4 pls.). The author describes the microscopical findings in smears of spleen and liver juice obtained by puncture. The appearance of the parasites was typical of *Leishmania donovani*, but numerous forms presented a vacuole between the nucleus and kinetoplast, such vacuolated forms being regarded as degenerate. It is noted that the parasites are intracellular when seen in sections of the organs, but are most frequently free in smears. This is due to fragility of the infected reticulo-endothelial cells (histiocytes), which rupture in the course of spreading the smear. C. A. H.

Mitochondria in Trypanosomes.—R. M. WOTTON ("A Study of the Mitochondria of Trypanosomes," *Quart. J. micr. Sci.*, 1940, **82**, 261–6, 1 pl.). The material on which this study was based were trypanosomes of the *lewisi* group (*Trypanosoma lewisi*, *T. duttoni*, *T. melophagium*, and *T. cruzi*) and of the *evansi* group (*T. equiperdum*), from the blood of the mammalian host, the intermediate host or culture. Blood or culture films were fixed by osmic acid and 95 p.c. ethyl alcohol, dried and stained by different methods. For the study of stages in the vector the insect was fixed and sectioned. The best results for the demonstration of mitochondria were obtained by staining with Altmann's acid fuchsin differentiated with aurantia. The mitochondria of all the trypanosomes examined appear as slender, rod-shaped bodies in the cytoplasm. They are few in number, mostly not exceeding twelve, and usually lie parallel to the long axis of the body. The mitochondria may be associated with granules of a fatty nature, and it is supposed that they are metabolic in function. In films treated by Kolatchev's method the "parabasal body" (=kinetoplast) of the trypanosome reveals its osmiophilic nature. Both this structure and the blepharoplast are surrounded by a clear zone. It is suggested that if the kinetoplast is analogous to the Golgi apparatus, then the clear zone may be regarded as a secretion product. C. A. H.

Immunization against Trypanosomes.—J. T. CULBERTSON ("Natural Transmission of Immunity against *Trypanosoma lewisi* from Vaccinated Mother Rats to their Young," *J. Parasitol.*, 1941, **27**, 75–9). Description of a method of immunization of young rats against *Trypanosoma lewisi* through the medium of vaccinated mothers. Infected rats were bled, the trypanosomes separated by centrifugation and suspended in 0·5 p.c. formolized saline. Female rats were given eight intraperitoneal injections of the vaccine at 3-day intervals, the dose being 0·1 c.c. per 15 gm. body weight of a suspension containing 4,300,000 organisms per 1 c.c. The females were mated while vaccination was in progress. When they delivered litters it was found that immunity acquired by vaccination was transmitted to the offspring by ingesting the milk of the mother. Immunity acquired through the placental circulation is lost within a few days after birth. C. A. H.

Infection of Chicks with Coccidial Merozoites.—P. P. LEVINE ("The Initiation of Avian Coccidial Infection with Merozoites," *J. Parasitol.*, 1940, **26**, 337–43). Description of experimental infection of chickens with the merozoites of five species of fowl *Eimeria*. The material containing merozoites was obtained by destroying infected birds before oocysts were developed. The mucosa of the infected part of the alimentary canal was scraped and suspended in saline, and the suspension was introduced (by pipette or catheter) into the appropriate site (crop, gizzard, intestine, cæca). It was shown that coccidial infection could be initiated with merozoites in all the five species. This method can also serve to isolate two species of coccidia in a mixed infection, advantage being taken of their development in different portions of the intestinal tract. C. A. H.

Schizogony in Fowl Plasmodium.—E. Beltran and R. Larenas ("El ciclo esquizogónico en *Plasmodium gallinaceum*, Brumpt," *Rev. Inst. Salubr. Enferm. trop.*, 1940, **1**, 291–309 : In Spanish with English summary). A study of the asexual cycle of development of the malaria parasite of domestic fowl, *Plasmodium gallinaceum*. The period of development was found to vary from 36 to 42 hours, the latter period being more frequent. Synchronicity was very high, with striking individual variations in the birds. The development is characterized by a high mortality of all stages of the parasite. C. A. H.

Effect of Piroplasm on Erythrocytes.—J. W. Landsberg and L. C. Eskridge ("The Erythrocytes in *Babesia* (*Piroplasma*) *Canis*," *J. Parasitol.*, 1940, **26**, 377–85). An account is given of the effect of a piroplasm, *Babesia canis*, on the red blood corpuscles of the dog. The infection causes anæmia, with a fatal termination, and it was found that the parasites were present in mature erythrocytes and had no predilection for reticulocytes. The presence or absence (in splenectomized animals) of the spleen did not appear to affect the issue. C. A. H.

Ciliates from China.—C. C. Wang ("Notes on some Fresh-water Infusoria," *Sinensia*, 1940, **11**, 11–32, 7 figs.). Description of seven new ciliates from Chinese fresh waters : *Prorodon microstomium* sp.n., *Amphileptus medius* sp.n., *Lionotus*. (*Hemiophrys*) *lineatus* sp.n., *Dileptus dimorphus* sp.n., *Glaucoma elliptica* sp.n. (Holotrichida) ; *Strongylidium crepidatum* sp.n., *Urostyla pseudomuscorum* sp.n. (Hypotrichida). C. A. H.

New Ciliate from Worm.—G. A. Noble ("*Trichodina urechi* n.sp., An Entozoic Ciliate from the Echiuroid Worm, *Urechis caupo*," *J. Parasitol.*, 1940, **26**, 387–405, 4 pls.). Description of a new peritrichous ciliate, *Trichodina urechi* sp.n., from the midgut of the echiuroid worm, *Urechis caupo*, found off the coast of California. The body is turban-shaped, with two concentric ciliary rings arising from the edge of the aboral suckring cup. A thin velum is situated median to the inner aboral ciliary ring. In the wall of the aboral cup are situated two supporting rings, and the oral zone is formed by a single spiral row of cilia. The cytopharynx contains two rows of cilia, one of which represents membranelles. A semi-circle of cilia lies on the floor of the aboral cup. Macronucleus is H-shaped. There is a single non-contractile vacuole, and a neuromotor system connected with a motorium. An account is given of the division process. C. A. H.

Conjugation in Paramecium.—V. Tartar and T.-T. Chen ("Mating Reactions of Enucleate Fragments in *Paramecium bursaria*," *Biol. Bull.*, 1941, **80**, 130–8, 4 figs.). A comparative study was made of the mating in two races of *Paramecium bursaria*, belonging to two different mating types in which the following reactions were examined : (*a*) Between two whole animals ; (*b*) Between nucleate fragments and whole animals ; (*c*) Between enucleate fragments and whole animals ; and (*d*) Between enucleate fragments only. When whole animals mated a flattening or indentation became apparent at the point of union. Nucleate fragments reacted positively with whole animals belonging to the other race, after which an intimate fusion as in normal conjugation took place. Enucleate fragments gave the mating reaction with whole animals of the other race, but did not respond to whole animals of the same race. However, the union between enucleate fragments and whole animals was not followed by intimate fusion. Mating reactions also occurred between enucleate fragments provided they belonged to different races. C. A. H.

BOTANY.

(*Under the direction of* J. RAMSBOTTOM, *O.B.E.*, DR.SC.)

Cytology.

Effect of X-rays on Chloroplasts of Polypodium aureum.—L. KNUDSON ("Permanent Changes of Chloroplasts induced by X-rays in the Gametophyte of *Polypodium aureum*," *Bot. Gaz.*, 1940, **101**, 721–58, 46 text-figs.). Changes were noted in the chloroplasts of protonemata and prothallia of *Polypodium aureum* after X-radiation of the spores from which they were derived. The spores will stand X-ray dosages as high as 30,000 *r* units without diminution of their percentage of germination, but in many of the prothallia thus derived the chloroplasts were abnormal in shape, size, or arrangement. Some of these abnormal chloroplast-types are transmitted to the sporophyte as a result of sexual reproduction, and retransmitted to the new generation of gametophyte. I. M. L.

Structure of Chloroplasts in Pteridophytes.—A. YUASA ("Studies in the Cytology of Pteridophyta. XVIII. The Structure of the Chloroplast of some Pteridophytic Plants," *Jap. Journ. Bot.*, 1940, **10**, 465–75, 7 text-figs.). The chloroplasts of certain species of *Selaginella, Adiantum, Polystichum, Dryopteris, Athyrium, Leptogramma*, and *Isoetes* were studied, both in living and in stained material. The chloroplast appears to be composed of a thin external membrane, an inner ground substance, and a network of chlorophyll, apparently in the form of granules connected by threads. The ground-substance filling up the interior of the chloroplast is of low viscosity. When division of a chloroplast is about to take place, a hyaline area appears in the central part of the network, and in this portion constriction finally brings about division into two. Starch grains were found in various parts of the chlorophyll network. I. M. L.

Induced Failure of Cytokinesis in Zea Mays.—G. A. LEBEDEFF ("Failure of Cytokinesis during Microsporogenesis in *Zea Mays* following Heat Treatment," *Cytologia*, 1940, **10**, 434–42, 26 text-figs.). As a result of heat treatment intended to induce chromosome doubling, failure of cytokinesis was observed in the anthers of maize during premeiotic and meiotic divisions. The division of the nuclei was not followed by division of the cells, so that multinucleate spores were formed, the size of the latter being approximately proportional to the number of nuclei present in them. I. M. L.

Golgi Material in Plants.—LAURA J. NAHM ("The Problem of Golgi Material in Plant Cells," *Bot. Review*, 1940, **6**, 49–72). A comprehensive summary of the literature on the occurrence in plants of the structures discovered by Golgi in animal cells in 1898 and named after him. Their morphology, terminology, methods of demonstration, chemical composition, and possible functions are discussed. Most recent workers are not inclined to regard Golgi bodies as morphological entities. I. M. L.

Mitochondria in Plants.—E. H. NEWCOMER ("Mitochondria in Plants," *Bot. Review*, 1940, **6**, 85–147). The literature on mitochondria in plants is here surveyed in detail, and it is seen that considerable conflict of opinion exists on many points regarding these structures. This has arisen chiefly through the tendency to accept as valid a number of appearances observed after chemical fixation without taking into account the possible physiological and morphological reactions induced by the chemical substances used. I. M. L.

Does Amitosis occur in Plants? J. McA. KATER ("Amitosis," *Bot. Review*, 1940, **6**, 164–80). In this discussion of the phenomenon of amitosis the plant and animal kingdoms are considered conjointly. Amitosis may be either reproductive or non-reproductive, although it appears that reproductive amitosis occurs only in ciliates. The nuclear divisions in yeasts do not appear to be amitotic, but this point is still somewhat unsettled; the ascus division is certainly mitotic. Divisions in the tapetal tissue of phanerogams are now recognized to be truly mitotic, and the only possible remaining record for plants is a somewhat doubtful observation by Komuro (1932) on the nuclear divisions in coal tar tumours in *Vicia Faba*. The Cyanophyceæ and bacteria are not here considered, because of the difficulty in correlating their internal structure with that of other cellular organisms.
I. M. L.

Effect of Salt Solutions on Chromonema Spirals.—M. SIGENAGA ("Artificial Uncoiling of the Chromonema Spirals with Neutral Salt Solutions," *Jap. Journ. Bot.*, 1940, **10**, 383–6, 1 pl.). Various chemicals have been found to induce an artificial uncoiling of the chromonema spirals of meiotic chromosomes in *Tradescantia reflexa*. Solutions of neutral salts, such as sodium chloride and potassium nitrate, are found to produce the same effect, which may be put to good use in the investigation of the problem of chromosome structure. I. M. L.

Cytological Effects of Neutron Irradiation.—Y. NISHINA, Y. SINOTÔ, and D. SATÔ ("Effects of Fast Neutrons upon Plants, III. Cytological Observations on the Abnormal Forms of *Fagopyrum* and *Cannabis*," *Cytologia*, 1940, **10**, 458–66, 16 text-figs.). As described in a previous paper, plants of *Fagopyrum esculentum* and *Cannabis sativa* grown from seeds which had been subjected to bombardment by fast neutrons were found to show various abnormalities, such as irregular or variegated foliage, fasciated stems, dwarf habit, abnormal flowers, etc. Abnormal chromosome behaviour appears to be rare in aberrant foliage and fasciations, indicating a relation to plastid alteration or possibly gene mutation, but the dwarf plants and those with irregular flowers were found to show certain peculiarities in the meiotic divisions (doubling of chromosomes, translocations, etc.), often followed by a shrivelling up of the quartets of pollen grains. I. M. L.

Meiosis in Diœcious Plants.—H. W. JENSEN ("Further Studies on Sex-linked Chromosome Abnormalities," *Cytologia*, 1940, **10**, 443–9, 16 text-figs.). In the diœcious plants *Xanthorrhiza apiifolia* and *Chamaelirium luteum* the meiotic divisions were found to be completely normal, without any trace of inequality among the chromosomes which might be interpreted as a sex-chromosome complex. The former species is monotypic and the latter is one of two comprising the genus. These facts are in agreement with the author's view that peculiar sex chromosomes are meiotic abnormalities brought about by previous hybridization between species, and are hence not to be found in monotypic species. The haploid chromosome number appears to be 15 in *X. apiifolia* and 12 in *C. luteum*. The author also studied the way in which the meiotic chromosomes originate in *Smilax herbacea*,

and made the observation that no free chromatids were present during the prophase of meiosis. He is therefore led to doubt the conventional hypothesis of active pairing of chromosomes at this stage, and believes that it is not until after the longitudinal splitting of the continuous spireme that segmentation into the haploid number of chromosomes (13) takes place. I. M. L.

Cytology of Desmodium and Lespedeza.—J. Oran Young ("Cytological Investigations in *Desmodium* and *Lespedeza*," *Bot. Gaz.*, 1940, **101**, 839–50, 43 text-figs.). *Desmodium* and *Lespedeza* are two closely related genera of Leguminosæ (subfam. Papilionatæ). The cytological investigation carried out by the author shows that in twenty-four species of *Desmodium* the haploid chromosome number is 11, and that in *Lespedeza* it is apparently 11 in one and 10 in ten of the eleven species studied. No karyological abnormalities were observed. I. M. L.

Cytological Studies in Calceolaria.—K. V. Srinath ("Morphological and Cytological Studies in the Genus *Calceolaria*. Part II. Meiosis in Diploid and Aneuploid Calceolarias," *Cytologia*, 1940, **10**, 467–91, 126 text-figs.). The chromosome constitution during meiosis was studied in four species of *Calceolaria*, of which three are diploid (*C. Clibrani*, *C. dentata*, and *C. Banksii*) and one aneuploid (*C. mexicana*). In the diploid species 2n is 18, and in *C. mexicana* 2n is 60. The nucleolus in the diploids becomes associated with one bivalent at diakinesis, while at the corresponding stage in *C. mexicana* it takes on two. Multivalents and rings or chains of four and six chromosomes were observed in the aneuploid species, but are rare in the diploids. There is reason to suspect that allopolyploidy prevails in the species with 2n=18, the basic chromosome number being 4. I. M. L.

Chromosomes of Bumelia lanuginosa.—W. L. Brown and R. B. Clark ("The Chromosome Complement of *Bumelia lanuginosa* and its Phylogenetic Significance," *Amer. Journ. Bot.*, 1940, **27**, 237–8). *Bumelia lanuginosa* (Sapotaceæ) was found to possess twenty-four somatic chromosomes. This fact makes it seem probable that the Sapotaceæ are more closely allied to the Styracaceæ than to the Ebenaceæ. I. M. L.

Cytological Studies in Gramineæ.—G. L. Church ("Cytotaxonomic Studies in the Gramineæ *Spartina*, *Andropogon*, and *Panicum*," *Amer. Journ. Bot.*, 1940, **27**, 263–71, 33 text-figs.). The 2n chromosome numbers were determined for members of the grass genera *Spartina* (nine species and four varieties investigated), *Andropogon* (nine species and eight varieties investigated), and *Panicum* (one species and one variety investigated). In *Spartina patens* the typical species is tetraploid, but its variety *juncea* is hexaploid in the northern and octoploid in the southern areas of its extension. Similar intraspecific chromosome differences were detected in *S. alterniflora* and *S. pectinata*. Diploid, tetraploid, hexaploid, and decaploid chromosome numbers were found in the species and varieties of *Andropogon* studied. *Panicum virgatum* on the Atlantic coast of N. America is tetraploid, while forms occurring in the western states are sometimes octoploid. I. M. L.

Cytology of the Genus Carex.—H. A. Wahl ("Chromosome Numbers and Meiosis in the Genus *Carex*," *Amer. Journ. Bot.*, 1940, **27**, 458–70, 2 pls., 1 text-fig.). The author has determined the chromosome numbers of 107 species and five hybrids of *Carex*. The haploid numbers form an aneuploid series ranging from 13 to 56. It is possible that the aneuploidy has been derived from euploid series with different basic numbers, of which 7 appears to be the most frequent. There are also 5- and 8-chromosome series. Sometimes the chromosomal constitution was found to vary within the same species; this indicates a somewhat high degree of polyploidy to

offset the lethal effects of this variation. Occasionally (particularly in the hybrids) tri- or quadrivalents were found. As a family, the Cyperaceæ appear to show three unique cytological features, namely: (*a*) The disintegration of three microspore fundaments in each tetrad; (*b*) The aneuploid series of chromosome numbers; and (*c*) Certain peculiarities in the behaviour of homologous chromosomes during meiosis. These peculiarities consist mainly in the splitting of the chromosomes during the first reduction division, the actual halving in number being apparently deferred until the second. I. M. L.

Cytology of Mosaic-seeded Maize.—FRANCES J. CLARK and F. C. COPELAND ("Chromosome Aberrations in the Endosperm of Maize," *Amer. Journ. Bot.*, 1940, **27**, 247–51, 11 text-figs.). Certain strains of maize having a high ratio of variegated seeds were investigated cytologically and found to show varying percentages of aberrations in the mitoses of their nuclei (lingering bridges, fragmentations, etc.). It is possible that these abnormalities are correlated with the physiologic changes in the endosperm of the seed. I. M. L.

Structure of Chromosomes.—J. IWATA ("Studies on Chromosome Structure. I. Spiral Structure of Chromosomes in *Trillium Smallii* Maxim."; "II. The attachment Chromomeres in the Meiotic Chromosomes," *Jap. Journ. Bot.*, 1940, **10**, 365–73, 2 pls., and 375–82, 1 pl., 15 text-figs.). In the first of these studies, the chromosome structure in *Trillium Smallii* during meiosis and pollen tetrad formation was investigated. Double coiled structure of the chromosomes makes its appearance in late diakinesis, and persists into the heterotype metaphse, in which ten gemini appear. Tertiary splitting is absent during the metaphase of both hetero- and homotypic divisions, but is visible at the other stages. A hyaline chromosome sheath was occasionally observed in the telophase. In the interval between the hetero- and homotypic divisions a loosening of the chromonema spirals takes place, and the structure tends to become reticulate, but the double coiled form persists more or less distinctly, and continues to be clearly recognizable in the homotype metaphase and anaphase. The second continuation deals with the attachment chromomeres in *Trillium Smallii* and *Lilium tigrinum*. These small bodies are visible on the chromosomes from early metaphase I up to the completion of the homotypic division. Trivalent chromosomes have six, bivalent chromosomes four, and univalent chromosomes two attachment chromomeres. During the telophase II in *Trillium*, the number of granules varies between seven and ten. As a rule the chromomeres are globular or oval, but double, dumb-bell-shaped granules occasionally occur. They react positively to Feulgen's nucleal test. Traction cones extending from the chromomeres were visible in both hetero- and homotypic divisions after suitable fixation. I. M. L.

Behaviour of Chromonemata in Mitosis.—Y. KUWADA and T. NAKAMURA ("Behaviour of Chromonemata in Mitosis. IX. On the Configurations assumed by the Spiralized Chromonemata," *Cytologia*, 1940, **10**, 492–515, 12 text-figs.). The process of nuclear division was observed in living staminate hairs of *Tradescantia reflexa* mounted in liquid paraffin and aqueous media. During division the chromonemata appear to be spiralized, either in a truly spiral or a twisted manner, the two combinations being mutually transformable. In the water medium the prophasic nucleus was seen to revert directly to the resting stage without passing into metaphase; this "abbreviated mitosis" is comparable to endomitosis. When an "abbreviated" mitosis is followed by a true mitosis, chromosome splitting appears to occur twice in the process of nuclear division, but this is not really so.

The question of the validity of certain commonly accepted conceptions of chromosome structure is discussed on the basis of these observations on living material.
I. M. L.

Cytological Relationships of Crassulaceæ.—J. T. BALDWIN, Jr. ("Cytophyletic Analysis of certain Annual and Biennial Crassulaceæ," *Madroño*, 1940, **5**, 184–92, 1 pl.). The Crassulaceæ are chiefly perennial, but some species are annual or biennial. Of these, the following have been studied cytologically and the chromosome numbers determined : *Sedum pusillum* Michx. ($2n=8$) ; *S. stellatum* L. ($2n=10$) ; *S. Nuttallianum* Raf. ($2n=20$) ; *S. annuum* L. ($2n=22$) ; *Diamorpha cymosa* (Nutt.) Britt. ($2n=18$) ; *Sedella pentandra* Sparsm. ($2n=18$) ; *S. pumila* (Benth.) Britt. & Rose ($2n=18$) ; and *S. Congdoni* (Eastw.) Sharsm. ($2n=18$). Thus the genera *Diamorpha* and *Sedella* show in their chromosome numbers a departure from the customary basic 4- and 5-chromosome systems of *Sedum*, a fact which confirms their separate generic status. I. M. L.

Cytology of Cruciferæ.—J. T. BALDWIN, Jr., and J. M. CAMPBELL ("Chromosomes of Cruciferæ. I. *Descurainia*," *Amer. Journ. Bot.*, 1940, **27**, 915–17, 12 text-figs.). The chromosome numbers were determined for the following four species (including nine subspecies) of *Descurainia* (Cruciferæ) ; *D. Sophia* (L.) Webb ($2n=28$) ; *D. Richardsonii* (Sweet) Schulz, subsp. *viscosa* (Rydb.) Detl. ($2n=14$), subsp. *procera* (Greene) Detl. ($2n=28$), and subsp. *incisa* (Engelm.) Detl. ($2n=42$) ; *D. obtusa* (Greene) Schulz, subsp. *typica* Detl. ($2n=14$), and subsp. *brevisiliqua* Detl. ($2n=42$) ; *D. pinnata* (Walt.) Britt. ($2n=42$ in glandular specimens, 28 in non-glandular specimens), subsp. *glabra* (Woot. & Standl.) Detl. ($2n=28$), subsp. *intermedia* (Rydb.) Detl. ($2n=28$), subsp. *Nelsonii* ($2n=14$), and subsp. *filipes* (Gray) Delt. ($2n=14$). Thus it is seen that this genus has evolved on a 7-chromosome system, with occurrence of diploid, tetraploid, and hexaploid strains. Polyploid differences were found in some cases to coincide with distributional peculiarities.
I. M. L.

Cytology of Crocus.—G. N. PATHAK ("Studies in the Cytology of *Crocus*," *Ann. Bot.*, 1940, n.s. **4**, 227–56, 65 text-figs.). The following twelve species and two varieties of *Crocus* were examined cytologically and their chromosome numbers determined : *C. Olivieri* ($2n=6$) ; *C. zonatus* ($2n=8$) ; *C. aerius* var. "Gray Lady" ($2n=8$) ; *C. ochroleucus* ($2n=10$) ; *C. susianus* ($2n=12$) ; *C. pulchellus* ($2n=12$) ; *C. speciosus* ($2n=18$) ; *C. speciosus* var. *albus* ($2n=12$) ; *C. Tomasinianus* ($2n=16$) ; *C. Korolkowii* ($2n=20$) ; *C. Salzmannii* ($2n=24$) ; *C. sativus* ($2n=24$) ; *C. sativus* var. *Elwesii* ($2n=15$) ; and *C. Tournefortii* ($2n=30$). The morphology of the somatic chromosomes was studied, and was found to show considerable variation in size and position of constrictions. Nearly all the species possessed also satellites and nucleoli, which were seen to be variable in size and equal in number to the satellites at telophase. *C. speciosus* is a hexaploid form, while its var. *albus* is tetraploid, the basic haploid number being 3. A doubling of the chromosome complement in the vegetative parts of the plant was observed in *C. Olivieri* and *C. sativus* var. *Elwesii*, giving rise to chimæras showing fusion of tissues with cells and nuclei of uneven size. The meiotic division was investigated in *C. susianus* and *C. ochroleucus* ; ring-formation of chromosomes was observed in the former species. I. M. L.

Cytological Study of a Xanthophyceous Alga.—M. CHADEFAUD ("Un curieux élément cytologique chez une Xanthophycée," *Bull. Soc. Bot. France*, 1939, **86**, 190–200, 7 text-figs.). In the unicellular Xanthophyceous alga *Chara-*

ciopsis minuta Borzi was found a curious spherule-like body surrounded by a number of guttæ which could be intravitally stained and which showed a reaction with iodine similar to that of glycogen. The " spherule " is at first colourless, but gradually develops red carotin pigmentation in older individuals. This peculiar element, which has some resemblance to the trichocyst-bodies found in certain *Euglenæ*, undergoes division simultaneously with that of the cell. I. M. L.

Polynucleate Cells in Symphoricarpus.—D. PERSIDSKY (" Polynuclear Cells in Petals and Sepals of *Symphoricarpus racemosus* Michx.," *Journ. Bot. Acad. Sci. RSS Ukraine*, 1940, **1**, 139–42, 4 text-figs.; Russian with English résumé). Cells with up to twelve nuclei were found in the petals and sepals of *Symphoricarpus racemosus* (Caprifoliaceæ); they are elongated in form and considerably larger than the surrounding normal uninucleate cells of the parenchymatous tissue. The author considers that the occurrence of these polynucleate cells may lend itself for use as a taxonomic criterion. I. M. L.

Cytological Changes induced by Halogens.—Y. SINOTÔ and A. YUASA (" The Cytomorphological Effects of Halogens and Halogen-Salts on Plant Cells," *Bot. Mag., Tokyo*, 1940, **54**, 205–14, 23 text-figs.). Treatment of root-tip cells of *Vicia Faba* with aqueous solutions of the halogens (F, Cl. Br, I) and their salts was found to give rise to various cytological aberrations, such as irregular mitoses, vacuolization of nuclei, formation of clear halos round the nucleoli, destruction of the nuclear contents, etc. Even more susceptible to this treatment than root-tips of *Vicia Faba* were the pollen mother-cells of *Chrysanthemum* sp. I. M. L.

Sensitivity of Cells to X-rays.—K. SAX and C. P. SWANSON (" Differential Sensitivity of Cells to X-rays," *Amer. Journ. Bot.*, 1941, **28**, 52–9, 6 text-figs.). In *Tradescantia* the sensitivity to X-rays of different types of cell, as measured by the frequency of the chromosomic aberrations induced, was found to vary. The resting microspore nuclei were highly resistant to X-radiation, but during karyokinesis the sensitivity was found to increase greatly, reaching its maximum at a point just before mid-prophase. Haploid microspores are more sensitive than diploid. The greatest effect of X-radiation is on the microsporocytes, followed (in descending order of sensitivity) by the microspores, root-tip cells, and generative cells. Obviously the degree of flux and mobility of the chromosome-complex is the chief factor determining relative sensitivity to the influence of X-radiation. I. M. L.

Anatomy and Morphology.

Embryo Development.—H. B. TUKEY and F. A. LEE (" Growth and Development of the Embryo and Fruit of the Peach as affected by Ringing and Defoliation of the Branches," *Bot. Gaz.*, 1940, **101**, 818–39). Ringing and defoliation of Peach was carried out with a view to checking development of the fruit and to determining the effect upon embryo development. This experiment showed that limiting the supply of materials from outside the fruit led to decrease in amount or retardation of mobilization of materials to the endosperm, nucellus, and pericarp, while the embryo continued to increase in size. These findings supplement results of a previous paper, which showed that it is abortion of the embryo which induces early ripening of the fruit and not the reverse. F. L. S.

Adventitious Shoots.—H. F. Bain ("Origin of Adventitious Shoots in Decapitated Cranberry Seedlings," *Bot. Gaz.*, 1940, **101**, 872–81, 15 figs.). An account is given of the development of adventive shoots in severed cranberry hypocotyls. They originate in the epidermal cell layer. F. L. S.

Hyacinthus Propagation.—E. Naylor ("Propagation of *Hyacinthus* by Leaf Cuttings," *Bull. Torrey Bot. Cl.*, 1940, **67**, 602–7, 8 figs.). When green leaves of *Hyacinthus* spp. are kept in clean, moist sand they develop numerous bulblets. These originate by division of epidermal and sub-epidermal cells. Roots arise from parenchymatous cells lying near the vascular strands. F. L. S.

Structure of the Cell Wall in Roots.—H. V. Witsch ("Zum Feinbau der Zellwand in Wurzeln," *Planta*, 1939, **29**, 409–18). The phloem parenchyma cells of various types of root differ in the structure of their cell walls. In roots with no secondary thickening (*Poa*, *Phaseolus*) these cell walls show a persistent tubular structure. Roots with slight secondary growth in thickness (*Helianthus*) have a foliar structure. Contractile roots (*Bellis, Plantago, Rumex, Allium, Ornithogalum, Crocus, Narcissus, Zantedeschia, Iris*) have a fibrous structure. B. J. R.

Distribution of Lignin in the Cell Wall.—H. E. Dadswell and D. J. Ellis ("Contributions to the Study of the Cell Wall. II. An Investigation of Delignification using Thin Cross Sections of Various Timbers," *Journ. Coun. Sci. Ind. Res.* (Australia), 1940, **13**, 129–37). Cross-sections of various timbers were progressively delignified and tested for lignin at intervals. Progressive weakening of the staining reactions corresponded to the reduction in the lignin as revealed by treatment with 72 p.c. sulphuric acid. There was no definite indication that the cell wall lignin was removed before or after the lignin of the "middle lamella" zone; all results pointed to simultaneous removal, although the proportion of lignin in the "middle lamella" zone is much higher. B. J. R.

Vegetative Propagation of Norway Spruce.—N. H. Grace, J. L. Farrar, and J. W. Hopkins ("Vegetative Propagation of Conifers. VII. Outdoor Propagation of a November Collection of Norway Spruce Cuttings treated with Phytohormones, Cane Sugar, and an Organic Mercurial Disinfectant," *Can. J. Res.*, 1940, **18**, 566–77). The preparations were applied in talc dust. Indolylacetic acid and naphthylacetic acid were applied in three concentrations, 0, 1000, and 5000 p.p.m., alone and in combination with cane sugar and an organic mercurial disinfectant. Phytohormone treatment, except with the 5000-p.p.m. concentration of naphthylacetic acid, which was injurious, was generally beneficial. The admixture of peat in equal proportions with sand improved rooting and development of new growth. Both cane sugar and organic mercury affected a number of the responses, but the effects were comparatively small and depended for the most part on interactions with phytohormone treatments and media. Organic mercury increased the number of cuttings rooted by about 6 p.c. B. J. R.

Winter Growth in Vegetative Buds.—H. P. Bell ("Winter Growth in the Vegetative Buds of the Wagener Apple," *Can. Journ. Res.*, 1940, **18c**, 585–9). Measurements of the median longitudinal section of buds collected from September to March show that a slow but continuous growth in length within the bud proceeds throughout the winter months. B. J. R.

Wood Structure of American Verbenaceæ.—S. J. Record and R. W. Hess ("American Woods of the Family Verbenaceæ," *Trop. Woods*, 1941, **65**, 4–21). Thirteen of the fifteen American genera with arborescent species are described.

Vitex is the only genus with commercial possibilities as timber. The woods of *Recordia* and *Rehdera* are not available for study. The differences observed between genera are not distinct enough to permit making a reliable key on the basis of wood structure. B. J. R.

Effect of Phytohormone Treatments on the Rooting of Picea Cuttings.—N. H. GRACE and J. L. FARRAR ("Vegetative Propagation of Conifers. VIII. Effects of Media and Phytohormone Dust Treatments on the Rooting of Norway Spruce Cuttings," *Can. Journ. Res.*, 1940, **18**c, 591–8). Dormant Norway spruce cuttings collected in November were treated with talc dusts containing indolylacetic acid, planted in five media, including two different sands and mixtures of these with peat humus. While 1000 p.p.m. indolylacetic acid increased survival in sand and there were marked differences between media and phytohormone treatments, the differences between the media were the striking feature of the results. Mixtures of peat humus with sand were uniformly superior to sand only. There were also some differences between a fine and a coarse sand, when used either alone or in combination with peat. B. J. R.

Effects of Phytohormone Treatments on the Rooting of Taxus Cuttings.—N. H. GRACE and J. L. FARRAR ("Effects of Talc Dusts containing Phytohormone, Nutrient Salts, and an Organic Mercurial Disinfectant on the Rooting of Dormant *Taxus* Cuttings," *Can. Journ. Res.*, 1941, **19**c, 21–26). Dormant *Taxus cuspidata* cuttings were treated with talc dusts containing 1- and 2-γ-naphthylbutyric acid at concentrations of 0, 500, 1000, and 2000 p.p.m., each taken separately and in combination with 0, 0·1, 1, and 10 p.c. of a mixture of nutrient salts and 0 and 50 p.p.m. of ethyl mercuric bromide. The treatments had no effect on the number of rooted cuttings. The number and length of roots per rooted cutting were increased in proportion to the concentration of the phytohormone. For most of the other criteria the results were injurious. Organic mercury failed to have any significant effects. B. J. R.

Effects of Phytohormone Treatment on the Rooting of Dahlia Cuttings.—N. H. GRACE ("Effects of Talc and Phytohormone Treatment on the Rooting of *Dahlia* Cuttings," *Can. Journ. Res.*, 1941, **19**c, 40–41). Groups of *Dahlia* cuttings, untreated, talc treated, and treated with talc containing various concentrations of naphthylbutyric acid, were propagated in sand. All the untreated cuttings died. Those treated with talc alone suffered 4 p.c. mortality. Phytohormone treatment increased the number of roots per rooted cutting, but increased the average mortality to 23 p.c. There were no significant differences in the effects of the various concentrations of phytohormone. B. J. R.

CRYPTOGAMIA.

Pteridophyta.

Germination in Hymenophyllaceæ.—ALMA G. STOKEY ("Spore Germination and Vegetative Stages of the Gametophytes of *Hymenophyllum* and *Trichomanes*," *Bot. Gazette*, 1940, **101**, 759–90, 35 figs.). An account of the stages of germination in seven species of *Hymenophyllum* and four of *Trichomanes*, with a discussion of the triradiate habit of the germlings, the question of the ancestry of the Hymenophyllaceæ, the ribbon-like outgrowths of the gametophyte of *Hymenophyllum*, and the filamentous outgrowths in that of *Trichomanes*; also the incompleteness of our knowledge of spore germination in these genera. A. G.

New Guinea Ferns.—A. H. G. Alston ("Undescribed Ferns from New Guinea," *Journ. of Bot.*, 1940, **78**, 225–9). Descriptions of eleven new species of ferns from the collections made by the following four travellers in New Guinea: (1) C. E. Carr (Owen Stanley Range: 500 specimens); (2) Miss L. E. Cheesman (Owen Stanley and Torricelli Ranges, Cyclops Mts., Waigell, Japan: 500 specimens); (3) Mrs. M. S. Clemens (Sattelberg: 1500 specimens); (4) Prof. Pulle (Dutch New Guinea: 250 specimens). A. G.

Welsh Ferns.—H. A. Hyde and A. E. Wade ("Welsh Ferns: a Descriptive Handbook," Cardiff: National Museum of Wales, 1940, x+131, 67 figs., 10 pls.). A handbook to the Welsh ferns, with new descriptions, keys, and figures. Brief accounts of the British species which do not occur in Wales are also included. The scheme of classification followed is that of Christensen's *Filicinæ* (1938). A short account of fern evolution and notes on family relationships are given on the lines laid down by Prof. F. O. Bower in his work, "The Ferns (*Filicales*) (1923–28)." The number of species native to Wales is forty: eleven of these are cosmopolitan, fourteen circumpolar, and ten have a restricted distribution. A. G.

Bryophyta.

Exormotheca.—Sultan Ahmad ("Morphological Study of *Exormotheca tuberifera*," *Bot. Gazette*, 1940, **101**, 948–54, 25 figs.). *Exormotheca tuberifera* was described by Kashyap in 1914 (*New Phytologist*, **13**, 308–12). A more detailed account of its structure is now given, based on material collected at Simla. A striking character of the thallus is the presence of tubers formed by a thickening of the apex or of modified shoots on the ventral surface; these serve for vegetative reproduction. Apical growth of the thallus takes place by divisions from a single cell. The antheridia develop in the same manner as in other Marchantiales, and arise in the mid-dorsal groove behind the female receptacle. The receptacle, on which the archegonia are borne, contains a layer of air chambers which open to the outside by simple pores. In the archegonium cell divisions occur in the venter even before fertilization, and result in the formation of a four-layered calyptra. The early development of the sporophyte shows the octant type of embryo. The apical cap of the capsule is single-layered, with small elater-like cells attached to it. The capsule dehisces by detaching the apical cap and splitting the side wall into four irregular valves. A. G.

Surrey Hepatics.—E. C. Wallace ("The Hepatics of Surrey," *Journ. of Bot.*, 1940, **78**, 257–62). A list of eighty-one species and six varieties of hepatics collected in Surrey in the last forty years. This total compares with seventy-four species recorded for Kent, fifty-six for Hampshire, and 119 for Sussex. In Sussex there are outcrops of sandstone rocks in the east and clayey soil in Ashdown Forest which favour the growth of additional and delicate species. Helpful notes on habitats, rarity, etc., are provided. A. G.

Riella.—R. H. Thompson ("A Second Species of *Riella* in North America," *Bryologist*, 1940, **43**, 110–11). Some notes on the submerged aquatic genus *Riella*. Of the seventeen species recorded for the world some are known only from the culture of dried mud sent from South Africa. *R. americana* Howe & Und. was collected in Texas and described in 1903; it was not found again until 1927. Another species, *R. affinis* Howe & Und., was also described in 1903, and was believed to be confined to the Canary Islands; but in May, 1939, it was found growing in great abundance in Lagunita, an artificial lake on the Stanford University campus

in California. During the winter this lake fills rapidly, becoming full in January. During May the water is drawn off for irrigation purposes, and the lake is empty by the middle of June, when it is possible to determine the distribution of the plant over the lake bed. It seems to grow most luxuriantly at a depth of 15–20 ft.; at shallower depths, 5 ft. and less, its growth is prevented by the action of waves. The difference between the two species is marked: *R. americana* is heterothallic, has smooth, unornamented archegonial involucres, and attains a height of about 1 in., whereas *R. affinis* is homothallic, has archegonial involucres ornamented with eight longitudinal wings, and does not exceed ½ in. in height. *Riella* is an erect plant reduced to a rib and a dorsal lamina; the archegonia occur on the rib and the antheridia in the lamina. A. G.

Gemmæ of Riella.—R. A. Studhalter and Martha Enna Cox ("The Gemma of *Riella americana*," *Bryologist*, 1940, **43**, 141–57, 28 figs.). An account of the origin and development of the gemmæ of *Riella americana*. The gemma originates as a single superficial cell on the ventral face of the axis. By two transverse divisions a filament of three cells is formed; the apical and basal cells divide no further, but the middle cell undergoes further division, and a curved filament of four to six cells is formed. Then by divisions perpendicular to the first cross walls an elongated unistratose plate of cells is produced, which overgrows and displaces to the ventral side both the original attachment cell and the apical cell now converted into a papillose mucilage cell. Rapid lateral growth at all points of the cell plate except in the middle zone results in the production of a bilobed unistratose mature gemma with numerous marginal mucilage cells; rhizoid initials are differentiated in the distal lobe; and all undifferentiated cells are well supplied with chloroplasts. The positions occupied on the axis of the plant by gemmæ, ventral scales, and lateral leaf scales are described. The gemmæ are regarded as the means by which the species quickly spreads itself in favourable situations.
A. G.

Plagiochila Sharpii.—H. L. Blomquist ("Another New Species of *Plagiochila* from the Southern Appalachian Mountains," *Bryologist*, 1940, **43**, 89–95, 1 pl.). Description and figures of *Plagiochila Sharpii*, a new species collected at Whitewater River Falls, in North Carolina, by H. L. Blomquist in the summer of 1939, and in Eastern Tennessee by A. J. Sharp in 1938; and a careful consideration of its possible affinities in the different systems of classification of the species of *Plagiochila* put forward by Spruce, Stephani, Carl, Gottsche, Schiffner. A. G.

Surrey Mosses.—E. C. Wallace ("The Mosses of Surrey," *Journ. of Bot.*, 1941, **79**, 1–11, 17–25). A list of all the mosses recorded for Surrey, 294 species, and several varieties, with their distribution, and brief notes on their habitats, rarity, etc. Many habitats have been destroyed by the spread of urban building and by drainage. Sussex and Kent have each a larger total of species, but Hampshire's total is smaller. Surrey, with its bogs, is richer in Sphagna than are the neighbouring counties. The chalk downs produce the most species, and the lower greensand hills, the boggy heaths of the Bagshot sands, and the woods on weald clay provide many species, but the London clay does not support many mosses.
A. G.

Thallophyta.

Algæ.

Nomenclature of Diatoms.—RUTH PATRICK (" A Suggested Starting-point for the Nomenclature of Diatoms," *Bull. Torrey Bot. Club*, 1940, **67**, 614–15). A proposal that the year 1891 should be fixed as the starting-point for diatom nomenclature. It is the year in which Volume II of De Toni's " Sylloge Algarum "—the volume on Bacillariaceæ—began to be published. Here for the first time was provided a bibliography of the vast literature on diatoms which had so far appeared ; and here for the first time are brought together all the genera of diatoms, each with an adequate description, and accurately determined from a nomenclatural standpoint. It was not till the middle of the nineteenth century that the improvement of microscopes began to make a critical study of diatoms possible. Then schemes of classification began to take shape, and several monographs of families and of genera were prepared and published. Just as modern dates, long after that of Linnæus's " Genera Plantarum " (1753), have been chosen as starting-points for other groups of small algæ (Nostocaceæ, 1886 ; Desmidiaceæ, 1848 ; Oedogoniaceæ, 1900), so 1891 for the Bacillariaceæ has everything to commend it. A. G.

Cyclonexis and Synochromonas.—F. W. JANE (" Two New Chrysophycean Flagellates—*Cyclonexis erinus* and *Synochromonas elæochrus*," *Proc. Linn. Soc. London*, 1940, 152nd Session (1939–40), 298–309, 4 figs.). *Cyclonexis* is a rare and little known genus originally recorded from North America over fifty years ago and found there again recently (*Rhodora*, 1939, 19). The original species was *C. annularis* Stokes. A second species, *C. erinus*, is now described : it has larger cells, each containing a single chromatophore (not two). The cells occur in ring-like colonies of 6–30 laterally attached individuals ; each cell bears two flagella, one long, straight, and undulating gently, the other shorter, curled, and lashing violently. The organism is very sensitive to changes of environment and soon breaks down ; hence it has to be studied in the living state, and to make accurate drawings of it is difficult. However, several figures of it are provided by the author. Material was collected in a *Sphagnum* swamp in Hertfordshire in May and June, 1937. The other genus, *Synochromones*, was described by Korshikov in 1929, with one species, *S. pallida*. A second species, *S. elæochrus*, has now been found in a pond in Middlesex, and is described and figured. The differences between the two species are shown in parallel columns. Briefly they are that the colonies of *S. elæochrus* are not embedded in mucilage ; the cells are symmetrical and have a rounded anterior end ; the chromatophores are large and not waisted ; the nucleus is large. A. G.

Mauritian Algæ.—F. BØRGESEN (" Some Marine Algæ from Mauritius. I. Chlorophyceæ," *K. Dansk. Vidensk. Selsk. Biol. Medd.*, 1940, **15**, no. 4, 1–81, 26 figs., 3 pls.). The first part of an account of the marine algæ of Mauritius, comprising fifty-six species of Chlorophyceæ collected by R. E. Vaughan, Th. Mortensen, N. Pike, and others. Six new species and one new genus are described ; figures and critical notes are appended to several other species. A bibliography is added. A. G.

Stigeoclonium.—MARGARET A. P. MADGE (" Zoospore Formation in a Species of *Stigeoclonium*," *New Phytologist*, 1940, **39**, 277–82, 1 pl.). An account of the cultivation of a species of *Stigeoclonium*, collected near Runnymede, and of observations made on the production and discharge of zoospores. The latter bear four flagella, which become active before the discharge of the zoospores ; and the

discharge is brought about by the rapid lengthening and enlarging of the enclosing wall which finally disappears and leaves the zoospores free. A. G.

Enteromorpha.—V. J. Chapman ("Some New Varieties of *Enteromorpha* and a New Species of *Monostroma*," *Journ. of Bot.*, 1940, **78**, 263–6.) Holding that the genus *Enteromorpha* is composed of a few highly polymorphic species rather than of numerous species with few variations, the author describes a number of novelties as varieties of existing species: namely, two new varieties of *E. clathrata* from South Africa and California respectively; two of *E. prolifera* from South Africa and New Zealand; three of *E. procera*, one being from California, Norway, and New Zealand, another from Jamaica, New Zealand, and Java, and the third from New Zealand. He also gives a description of *Monostroma Lindaueri*, a new species from Bay of Islands, New Zealand. A. G.

Carpophyllum.—A. E. Else Dawson ("Studies in the Fucales of New Zealand. II. Observations on the Female Frond of *Carpophyllum flexuosum* (Esp.) Grev.=*Carpophyllum phyllanthus* (Turn.) Hook. & Harv.," *New Phytologist*, 1940, **39**, 283–302, 4 figs.). *Carpophyllum flexuosum* is typically Sargassaceous in habit; male and female fronds are practically indistinguishable. The apical cell is a three-sided truncated pyramid; in the receptacles the conceptacles are spirally arranged in three vertical rows. The conceptacles originate from a sunken epidermal cell which divides into two, the upper "tongue-cell" forming a short filament, and the lower "initial cell" acting as an apical cell. The centre of the floor of the conceptacle is occupied by a sterile column of concrescent hairs branched at the end (possibly the vestigial remains of antheridia of a monœcious ancestor). The oogonia appear to be formed in threes, spirally arranged in the conceptacle; they are normally uninucleate, are supported on a stalk cell, and mature before extrusion. The oogonial wall becomes differentiated into three layers; the mesochiton forms a stalk, by whose unfolding the oogonial contents become extruded and anchored. Some of the factors controlling this extrusion are discussed. After extrusion, cytological maturation soon becomes completed, and the seven supernumerary nuclei only degenerate after fertilization, by disappearing in the cytoplasm. The "oogonium" is interpreted as a megasporangium producing a syncytial gametangium equivalent to four two-celled gametophytes. A multicellular sporeling of the *Sargassum* type develops within the mesochitinous sheath. Living in calm water the species maintains a well-developed reproductive economy, with cross-fertilization and the production of solitary attached oospores, which begin their germination in close relation to the parent plant. A. G.

Fungi.

New Uncinula.—F. J. Seaver ("A New Powdery Mildew," *Mycologia*, 1940, **32**, 649–52, 1 fig.). A species of *Uncinula*, hitherto undescribed, was found on leaves of *Jaborosa integrifolia* in the greenhouse of the New York Botanical Garden. It is characterized by appendages four to five times the diameter of the perithecium. F. L. S.

Morenœlla.—E. S. Luttrell ("*Morenœlla quercina*, cause of Leaf Spot of Oaks," *Mycologia*, 1940, **32**, 652–68, 13 figs.). *Morenœlla quercina*, which causes a leaf spot on oaks, is fairly widespread in the South-Eastern United States. It occurs on various species of oak in Duke Forest, but not on *Q. alba* and *Q. stellata*

in this locality. The development and structure of the fungus was studied and is described in detail. A spermogonial stage, hitherto unrecorded, is also described.
F. L. S.

Myriangium.—J. H. MILLER ("The Genus *Myriangium* in North America," *Mycologia*, 1940, **32**, 587–601). An account of the four species of *Myriangium* occurring in America. *M. Duriæi* and *M. asterinosporium* are the commonest species, the former occurring on nearly every black gum bearing scale insects, and the latter on almost every hawthorn parasitized by scales. While both have also been found on other hosts they have not been observed to interchange hosts.
F. L. S.

Essex Discomycetes.—W. D. GRADDON ("Discomycetes Recorded in Essex," The *Essex Nat.*, 1940, **27**, 19–29). A list is presented of 135 species with short notes on seventeen species recently recorded. F. L. S.

British Smuts.—K. SAMPSON ("List of British Ustilaginales," *Tr. Br. Myc. Soc.*, 1940, **24**, 294–312). The seventy names appearing in this list of British Ustilaginales are arranged under the three families Ustilaginaceæ, Tilletiaceæ, and Graphiolaceæ. F. L. S.

Indian Smuts.—B. B. MUNDLSUR ("A Second Contribution towards a Knowledge of Indian Ustilaginales," *Tr. Br. Myc. Soc.*, 1940, **24**, 312–37). Seventy collections of Indian smuts were examined and considered to include twenty-five species of which six are described for the first time. F. L. S.

Colombian Rusts.—F. D. KERN and H. W. THURSTON ("A Further Report on the Uredinales of Colombia," *Mycologia*, 1940, **32**, 621–30). Notes on several species of rusts from Colombia and an account of two species new to science. These are *Puccinia immensispora*, with teleutospores $31–40 \times 125–170\mu$, and occurring on a host identified as *Diplostephium* (?) and *P. liabicola* occurring on *Liabum* sp.
F. L. S.

Tropical Rusts.—G. B. CUMMINS ("Descriptions of Tropical Rusts," *Bull. Torrey Bot. Cl.*, 1940, **67**, 607–14, 2 figs.). An account is given of nine new species of rusts and notes are made on a few other interesting species. F. L. S.

Florida Lactariæ.—H. C. BEARDSLEE and G. S. BURLINGHAM ("Interesting Species of Lactariæ from Florida," *Mycologia*, 1940, **32**, 575–87, 4 figs.). Seven species of *Lactarius* are described as new to science. They were collected in the autumn and winter in Orange and Seminole counties, Florida. Three of the figures are photographs of the species, the fourth consists of drawings of the spores.
F. L. S.

New Pistillaria.—R. E. REMSBERG ("A New Species of *Pistillaria* on Rice Straw," *Mycologia*, 1940, **32**, 667–71, 1 fig.). Fruiting bodies of a *Pistillaria* developed when rice straw from Louisiana was kept in a moist chamber. It is described as new to science under the name *P. Oryzae*. F. L. S.

Aspergillus.—G. H. GOSSOP, E. YUILL, and J. L. YUILL ("Heterogeneous Fructifications in Species of *Aspergillus*," *Tr. Br. Myc. Soc.*, 1940, **24**, 337–45, 1 pl.). Conidia of two sorts were found in fructifications of *Aspergillus* species when grown in mixed culture. Such fructifications were found only when the cultures constituting the mixture were related, as different mutants. It is suggested that the dual composition of the heads may be due to mycelial anastomoses. F. L. S.

Mutations in Aspergillus.—R. M. WHELDEN ("'Mutations' in *Aspergillus niger* bombarded by Low Voltage Cathode Rays," *Mycologia*, 1940, **32**, 630–44, 4 figs.). Various strains of *Aspergillus niger* resulted from the irradiation of spores of this fungus, and these remained constant through several asexual generations. The electrons apparently penetrated sufficiently to release energy in the nuclear zone, and hence the changes produced are regarded as mutations. The cytology of all the variants, with one exception, was similar. In the exception, a large form, double the normal number of chromosomes was observed. F. L. S.

Helminthosporium and Toxicity.—J. J. CHRISTENSEN and F. R. DAVIES ("Variations in *Helminthosporium sativum* induced by a Toxic Substance produced by *Bacillus mesentericus*," *Phytopath*, 1940, **30**, 1017–33, 4 figs.). *Bacillus mesentericus*, grown on artificial media, produced a substance which suppressed growth, increased conidial production, inhibited or retarded germination, and induced mutation in certain races of *Helminthosporium sativum*. The effect of different conditions on the potency of the toxic substance was also investigated.
F. L. S.

Plant Diseases.—W. C. MOORE ("New and Interesting Plant Diseases," *Tr. Br. Myc. Soc.*, 1940, **24**, 345–52, 2 pls.). An angular leaf spot of apple, a leaf spot of lettuce, and a *Pythium* disease of *Colchicum* bulbs are described. F. L. S.

Pittosporum Disease.—A. G. PLAKIDAS ("Angular Leaf Spot of *Pittosporum*," *Mycologia*, 1940, **32**, 601–9, 4 figs.). A species of *Cercospora* not previously described was found forming an angular leaf spot on *Pittosporum tobira* from New Orleans. It was isolated in culture and its pathogenicity was proved. F. L. S.

Host Specialization.—G. W. FISCHER ("Host Specialization in the Head Smut of Grasses, *Ustilago bullata*," *Phytopath.*, 1940, **30**, 991–1017, 2 figs.). Cross inoculations were made with forty-four collections of *Ustilago bullata*, including *U. bromivora* and *U. lorentziana*, which are not specifically separable either on a morphological or on a host specialization basis, from thirty-six species of grasses. Eight physiologic races were recognized. The most common race, widespread in the Pacific North-West on *Bromus tectorum*, was capable of infecting a number of species of *Agropyron*, *Elymus*, and *Festuca*. *Elymus glaucus* was the most susceptible of the economic species. F. L. S.

Take All.—E. M. TURNER ("*Ophiobolus Graminis* sacc. var. *Avenae* var. N., as the Cause of Take All or Whiteheads of Oats in Wales," *Tr. Br. Myc. Soc.*, 1940, **24**, 269–82). Outbreaks of Take All have been reported in recent years in oats, generally regarded as resistant to this disease. Isolates of *Ophiobolus* from oats in Wales were found to be very pathogenic to oats which are resistant to *O. graminis* from England. This Welsh form was studied in some detail and is regarded as a new variety, *O. graminis* sacc. var. *Avenae* E. M. Turner. F. L. S.

Heterothallism.—R. W. DAVIDSON ("Heterothallism in *Ceratostomella multiannulata*," *Mycologia*, 1940, **32**, 644–6). Crossing experiments showed that *Ceratostomella multiannulata* is heterothallic with two sex groups. F. L. S.

Dwarf Bunt.—C. S. HOLTON ("Preliminary Investigations on Dwarf Bunt of Wheat," *Phytopath.*, 1941, **31**, 74–82, 5 text-figs.). The reticulate chlamydospores of the dwarf-bunt race of *Tilletia Tritici* did not germinate under the treatments given, but the small number of smooth hyaline resting spores which are mixed with the reticulate spores often germinated. Infection tests, using a combination of

apparently monsporidial lines, produced characteristic dwarf-bunt disease. Hybrids were obtained between the dwarf bunt race and race 12 of *T. Tritici* and race 9 of *T. levis*. F. L. S.

Rhizoctonia.—E. L. Le Clerg ("Pathogenicity Studies with Isolates of *Rhizoctonia Solani* obtained from Potato and Sugar Beet," *Phytopath.*, 1941, **31**, 49–61, 2 figs.). Eighty-nine isolates of *Rhizoctonia Solani* obtained from lesions on the underground stems of old potato plants or from sclerotia on tubers were non-pathogenetic to sugar-beet roots. However, a few isolates from potato stolons infected in the spring rotted sugar-beet roots. The sugar beet isolates inoculated into potato were more aggressive to potato than the potato isolates used for comparison. Field observations showed that sugar-beet crops following potato were relatively free from root rot in contrast to sugar-beet following sugar beet. F. L. S.

Foot-rot of Stocks.—A. W. Dimock ("The *Rhizoctonia* Foot-Rot of Annual Stocks (*Matthiola incana*)," *Phytopath.*, 1941, **31**, 87–91, 2 text-figs.). One or more strains of a *Rhizoctonia* sp. were proved to cause the serious foot-rot disease of stocks in the Eastern States of America. Infection of plants up to two months old occurs at or just below the soil line. Other crucifers were also found to be susceptible to this fungus from stocks. Control was obtained by sterilizing soil with either steam or chloropicrin. F. L. S.

Pine Disease.—F. A. Wolf and W. J. Barbour ("Brown-Spot Needle Disease of Pines," *Phytopath.*, 1941, **31**, 61–74, 4 text-figs.). A brown-spot disease of pine needles, originally described as *Cryptosporium acicolum* Thüm., but now known as *Lecanostiata acicola* (Thüm.) Sacc., was found to be very destructive to seedlings and young trees of *Pinus palustris* in U.S.A. Its morphology, development, and taxonomy were studied. Genetic connection was established between the saprophytic perithecial stage, *Systremma acicola* (Dearn.) nov. comb. and the parasitic conidial stage. F. L. S.

Stereum of Hardwoods.—R. W. Davidson, W. H. Campbell, and R. C. Lorenz ("Association of *Stereum Murrayi* with Heart Rot and Cankers of Living Hardwoods," *Phytopath.*, 1941, **31**, 82–7, 1 fig.). Isolations of *Stereum Murrayi* (B. & C.) Burt. were made from several trees, *Betula lutea*, *Acer saccharum*, *A. rubrum*. Cultures were of slow growth, showing dense white mycelium, distinctive smell, and russet vinaceous to fawn colour of the agar. The fungus produces cankers and heart rot. F. L. S.

Larch Mycorrhiza.—J. E. How ("The Mycorrhized Relations of Larch. II. The Role of the Larch Root in the Nutrition of *Boletus elegans* Schum.," *Ann. Bot.*, 1941, **5**, 121–31). Experiments were made of growing *Boletus elegans* in cultures containing excised primary roots of European larch, Japanese larch, and Scots pine. A water soluble substance capable of stimulating the growth of the fungus was found in the roots of the two larches, but not in those of pine. F. L. S.

Indian Fungi.—Sultan Ahmad ("Higher Fungi of the Punjab Plains II," *Journ. Indian Bot. Soc.*, 1940, **18**, 169–77, 2 pls.). Of the fifteen fungi here described nine are new records for India. The fungi dealt with belong to the families Hysterangiaceæ, Lycoperdaceæ, Geasteraceæ, Sphaerobolaceæ, Nidulariaceæ, Phallaceæ, and Clathraceæ. F. L. S.

Lichens.

Chemistry of Lecanora gangaleoides.—T. J. NOLAN and J. KEANE ("The Chemical Constituents of Lichens found in Ireland. *Lecanora gangaleoides.*—Part 2," *Sci. Proc. Roy. Dublin Soc.*, 1940, **22**, 199–209). In a previous communication on the chemistry of *Lecanora gangaleoides* the authors showed that this lichen contains gangaleoidin, $C_{18}H_{14}O_7Cl_2$, as its main constituent and atranorin and chloratranorin as subsidiary constituents. A fourth substance of higher melting-point was isolated, and is now found to approximate in its chemical constitution to the formula $C_{26}H_{21}O_{10}Cl_3$. The presence of small amounts of arabitol, endococcin, and rhodophyscin was also demonstrated, and it was found that endococcin can be converted into rhodophyscin by boiling with acetic acid, the two substances being closely related and possibly isomeric. I. M. L.

Chemistry of Parmelia latissima.—T. J. NOLAN, J. KEANE, and V. E. DAVIDSON ("The Chemical Constituents of the Lichen *Parmelia latissima* Fée," *Sci. Proc. Roy. Dublin Soc.*, 1940, **22**, 237–9). A specimen of *Parmelia latissima* from Kenya was analysed, and found to contain four main constituents: lecanoric acid (predominant), a mixture of atranorin and chloratranorin, salazic acid, and *d*-arabitol. I. M. L.

Distribution of Cladoniaceæ.—H. SANDSTEDE ("Cladoniaceæ, III.," in: "Die Pflanzenareale," Jena, 1939, 4. Reihe, Heft. 8, 93–102, 10 maps). The conclusion of the cartographic studies of *Cladonia*-distribution published in two previous instalments in "Die Pflanzenareale." It deals with the group *Ochrophaceæ* of the subgenus *Cenomyce* of *Cladonia*, ninety-five species and several varieties being listed and their ranges entered on the maps. Other species not included in the cartographic section are enumerated briefly with mention of the salient characters of their distribution. I. M. L.

Epiphyllous Lichens.—A. OXNER ("Epiphyllous Lichens of the Caucasus in Respect to their General Distribution," *Journ. Inst. Bot. Acad. Sci. RSS Ukraine*, 1939, no. 21–22 (29–30), 307–21, 4 text-figs.; Russian with English résumé). The author argues that the term "epiphyllous" in regard to lichens is too wide in its current delimitation, and proposes its restriction to define only those crustaceous species which are confined to the surface of perennial leaves; these truly epiphyllous lichens are peculiar to tropical rain-forests and form a well-defined biological group in most cases also taxonomically distinct (Strigulaceæ, Pilocarpaceæ, Ectolechiaceæ). Five species of epiphyllous lichens found in the Caucasus are listed: *Byssoloma tricholomum* (Mont.) Zahlbr., *Catillaria Bouteillei* (Desm.) Zahlbr., *Phylloporina obsoleta* Oxn., n.sp., *Sporopodium caucasicum* Elenk. & Woronich., and *Strigula elegans* (Fée) Müll. Arg. The genus *Phylloporina* is new to the Caucasus; its world distribution, here shown on a map, shows it to be a relic of the ancient pre-Miocene tropical forest flora (Poltara flora), and the same applies to *Sporopodium caucasicum* and *Strigula elegans*. The paper concludes with a map showing the general geographical distribution of epiphyllous lichens. I. M. L.

Rhizocarpon simillimum found in Scandinavia.—A. SCHADE ("*Rhizocarpon simillimum* (Aazi) Lettau, eine für Skandinavien neue Flechte, in Gesellschaft interessanter Kümmerformen anderer Krustenflechten," *Svensk Bot. Tidskr.*, 1939, **33**, 347–65, 5 text-figs.). *Rhizocarpon simillimum*, new to Scandinavia, was found as a sparingly developed admixture in specimens from Sweden, Härjedalen, consisting of an intimate intermixture of very reduced states of the following species: *Rhizocarpon geographicum, R. superficiale, R. obscuratum, R. Hochstetteri, R. inter-*

situm, *Lecidea Dicksonii*, *L. plana*, *Sarcogyne simplex*, *Lecanora polytropa*, *Buellia sororia*, and *Aspicilia*-sp. Such reduced states, however, usually conserve the normal dimensions of the microscopic apothecial features, and for these it is proposed by the author to employ the term " f. *depauperata* " without citing the name of any authority. Where, on the other hand, such reduction does go hand in hand with production of smaller spores, as was found in the specimens of *R. intersitum*, it is suggested that the epithet " f. *minuta*," with author's name appended, should be applied. Consequently the reduced specimens dealt with here are described as *R. intersitum* f. *minutum* Schade, f.n.; their spores measure only $21–23 \times 10{\cdot}5–12{\cdot}5\mu$. Long periods of burial under heavy snow-falls are considered by the author to be the chief factor influencing the depauperation which is a feature of all the species making up the complex examined. I. M. L.

New Species of Lichens.—Bouly de Lesdain (" Notes Lichénologiques, XXXI," *Bull. Soc. Bot. France*, 1939, **86**, 81–4). The following new species are described: *Staurothele Sienæ* B. de Lesd. (Haly), *Endopyrenium Bajadanæ* B. de Lesd. and *E. Americanum* B. de Lesd. (both from New Mexico), *Psora Americana* B. de Lesd. (New Mexico), *Lecidea Michoacanensis* B. de Lesd. (Mexico), *Physma cataractæcola* B. de Lesd. (New Mexico), *Caloplacopsis Balansæ* B. de Lesd. (Paraguay), *Lecanora* (*Eulecan.*) *Genuensis* B. de Lesd. (Italy), *Platysma parmelioides* B. de Lesd. (E. Indies), and *Buellia Cacoti* B. de Lesd. (New Caledonia). A new form *albida* of *Physcia orbicularis* is also described from Italy, and supplementary descriptions are given of *Calicium Cubanum* B. de Lesd. and *Lecania erysibe* var. *grisea* (Flag.) B. de Lesd. I. M. L.

New and Interesting Lichens of U.S.S.R.—A. Oxner (" New for the U.S.S.R. and Little-known Lichens," *Journ. Bot. Acad. Sci. RSS Ukraine*, 1940, **1**, 101–9, 7 text-figs.; Russian with English résumé). The following new species of lichen found in various parts of the Soviet Union are published: *Thelocarpon Tichimirorii* Oxn. (E. Siberia, Yakut); *Aspicilia Grossheimii* Oxn. (Azerbaidzhan); *Ramalina kazakhorum* Oxn. (Kazakh); and *R. Kardakoræ* Oxn. (Primorskaya, Petrov Islands). Also a new form, *sorediosa* Oxn. of *Cetraria Braunsiana* (Müll. Arg.) Zahlbr., which species had not been previously recorded from U.S.S.R. Other species constituting new records for the Soviet Union are *Cetraria Asahinæ* Sato and *Lecanora pachycheila* Hue. The three last-mentioned lichens are interesting on account of their eastern Asiatic oceanic distribution. *Psora pulcherrima* (Vain.) Elenk., a rare Russian lichen, is reported from two additional stations. I. M. L.

Lichens from Central Asia.—A. Oxner (" Contribution to the Lichen Flora of Middle Asia," *Journ. Inst. Bot. Acad. Sci. RSS Ukraine*, 1939, no. 20 (28), 111–36, 6 text-figs.; Russian with English résumé). Taxonomic elaboration of several collections of lichens sent to the author from various parts of Central Asia —Turkmenia, Tiang-Shang, Fergana, Kazakh, Karsakpay, Dzhap-Arkin, and Alma-Ata. It comprises 109 species, of which the following are new to science: *Staurothele Larazenkoi* Oxn., *S. Levinae* Oxn., *Aspicilia lacteola* Oxn., *Placodium Kotovii* Oxn., *P. verruculiferum* Oxn., *Lecania bullata* Oxn., *Buellia kirghisorum* Oxn., and *Rinodina Melvillii* Oxn. A number of other species interesting for taxonomic or phytogeographic reasons are dealt with critically and in detail. I. M. L.

Lichens from Eastern Siberia.—A. Oxner (" Lichens of the Lena, Yana, Indigirka River Basins and Southern Pribaikalye, II " and " III," *Journ. Bot. Acad. Sci. RSS Ukraine*, 1940, **1**, 77–100 and 313–24; Russian with English résumé). Two continuations of the first part of the author's study of lichens

collected from Eastern Siberia, previously published in *Journ. Inst. Bot. Acad. Sci. RSS Ukraine* (1940). The first of these continuations deals entirely with the family Cladoniaceæ, of which thirty-five species belonging to the genera *Pilophoron*, *Bacomyces*, and *Cladonia* are listed ; in the second the remaining genus of Cladoniaceæ, *Stereocaulon*, and the families Umbilicariaceæ, Acarosporaceæ, Pertusariaceæ, and Lecanoraceæ are presented. Some of the species are of particular interest on account of their rarity. I. M. L.

Lichens of the New York Area.—G. G. NEARING (" Guide to the Lichens of the New York Area," Parts 5–8, *Torreya*, 1940, **40**, 9–18, 34–9, 110–7, 198–206, 56 text-figs.). Continuation of the author's popular account of the commoner lichens of the New York area, dealing with the genera *Sticta*, *Solorina*, *Nephroma*, *Peltigera*, and *Physcia* (the latter understood as embracing also *Anaptychia* and *Pyxine*). No keys to the species are given. The illustrations are simple, but express the habit of the various species well. I. M. L.

The Cladoniæ of New Jersey.—A. W. EVANS (" The Cladoniæ of New Jersey —Second Supplement," *Torreya*, 1940, **40**, 141–65). A second addition to the author's list of New Jersey *Cladoniæ* published in 1935. Three species are now added to the flora of this area : *C. vulcanica* Zolling, *C. nanodes* Robbins, and *C. gracilis* (L.) Willd., and also thirty-five forms. The total number of known species from the State of New Jersey is now forty-nine. I. M. L.

Cladonia alpestris found in Pennsylvania.—W. L. DIX (" *Cladonia alpestris* near Lake Shehawken," *Torreya*, 1940, **40**, 45). *Cladonia alpestris* was discovered near Lake Shehawken, in Wayne County, Pa. The record constitutes a link between the previously known stations in the New York area and Western Virginia. I. M. L.

New American Record for Cladonia floridana.—D. SMILEY (" Extension of Range of *Cladonia floridana*," *Torreya*, 1940, **40**, 45). Quotation from a letter by R. H. Torrey, dated 1937, in which the occurrence of *Cladonia floridana*, a species first described from Florida, is recorded in Maryland, Southern New Jersey, Long Island, Ellenville, N.Y., and Massachusetts. It is predominantly a coastal plain plant, but may on occasion inhabit higher altitudes (*e.gr.* 670 m. in the Shawangunks). I. M. L.

New Lecidea from Brazil.—A. W. HERRE (" A New Species of Lecidea from Brazil," *Madroño*, 1940, **5**, 235–6). Description of *Lecidea viçosensis* from Minas Geraes, Brazil, collected on the bark of a tree by Ynes Mexia in 1930. It belongs to the section *Biatora*. I. M. L.

Reproductive Phenomena in Dermatocarpon aquaticum.—R. B. STEVENS (" Morphology and Ontogeny of *Dermatocarpon aquaticum*," *Amer. Journ. Bot.*, 1941, **28**, 59–69, 84 text-figs.). The development of pycnidia and perithecia was studied in *Dermatocarpon aquaticum* by cytological methods. It was found that from each perithecial primordium at least one trichogyne is produced ; this trichogyne being an extension of one of the hyphal filaments making up the primordial coil, and eventually emerging on the upper surface of the thallus. It differs from the lower filaments in being thick-walled and devoid of cross-septa. In some of the preparations numerous pycnidiospores were found adhering to the exposed ends of the trichogynes, which degenerate before development of ascogenous hyphæ takes place. Ascus-formation is initiated by the formation of what appear to be typical " crosier " hyphæ of the ascomycetous type, each containing a fusion nucleus which by three consecutive divisions gives rise to the eight nuclei which are eventually embodied in the ascospores. I. M. L.

NOTICES OF NEW BOOKS

Fluorescence Analysis in Ultra-Violet Light.—By J. A. Radley and Julius Grant. 1939. Third Edition, revised and enlarged. xvi+424 pp., 21 text-figs., 28 plates. Published by Chapman & Hall, London, W.C.2. Price 25*s.* net.

That this book has reached a third edition within six years is evidence of the interest taken in a subject that in a scientific sense is somewhat limited in scope. The introductory chapters on the theory and technique are interesting in themselves ; they can be read with profit by those wishing to acquire a general knowledge of methods and applications. The actual production of ultra-violet light is the keystone of the whole subject ; the availability of a suitable light source often determines the limits of any work contemplated. This side of the subject is dealt with adequately, but there is not a great deal of space devoted to the practical side of the use of apparatus and of the methods needed for any particular application. Thus, the subject of fluorescence microscopy, which is of primary interest to the readers of the Journal of the Royal Microscopical Society, is treated so discursively that it is difficult to understand its purpose. Ultra-violet microscopy is referred to, but it is doubtful if the authors appreciate its aims, and apparently have no familiarity with the methods employed. Fluorescence is one of the hindrances to successful ultra-violet microscopy and is always likely to remain so until some hitherto unknown method is discovered. It follows that familiarity with the use of ultra-violet light for microscopical purposes is the best of all introductions to its application as a means of developing the use of fluorescence microscopy for any special purpose. The book is well produced and the references are numerous and sufficiently complete.

J. E. B.

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TRANSACTIONS OF THE SOCIETY.

1.—CONTRIBUTION TO THE KNOWLEDGE OF THE DIATOM GENUS *MASTOGLOIA*. 582.61

BY MANFRED VOIGT, F.R.M.S.

SIX PLATES.

THE Diatom genus *Mastogloia* has been subjected to a general survey by Dr. F. Hustedt (1930) in Rabenhorst's Kriptogamenflora. This work includes not only the European species, but all those forms which a careful study of existing publications and collections brought to the notice of the author, excluding only the fossil species and some few doubtful ones, about which too little information was available; it thus furnishes an up-to-date synopsis of great value upon which the following notes are mainly based.

The distinctive feature of the genus is the septate ring, divided into so-called loculi and placed between the connective zone and the valve surface, but the various other characteristics of the different species vary so widely, that some authors are tempted to ask whether the loculi have all the generic value which is usually attributed to them (A. Mann, 1907). On the other hand, in spite of this variation of structure, certain features frequently occur, which will often enable the student to recognize a *Mastogloia* frustule, even if the loculi have become detached or lost during the cleaning processes.

Some of the most striking of these features are: an undulatory more or less inclined, broad or complex raphe; a particularly intricate arrangement and form of the puncta or alveoli composing the surface marking; the presence of furrows, depressions, oculi, and other distinctive marks not so commonly met with in other naviculoid genera. As the loculi are in many cases, small and easily overlooked, particularly when the valve surface is strongly convex and heavily silicified all new forms exhibiting a combination of any of the

above-mentioned features, should be closely examined before being classed under any other genus. When examined dry, under a low magnifying power, reddish brown and yellow tints will be found to predominate and will facilitate the work of selecting *Mastogloia* frustules for individual mounts.

A careful examination of the septate ring, which can often, without great difficulty be separated from the valve, enables us to distinguish several types of loculi:

(1) Those consisting of simple loculi, usually few in number and of relatively large dimensions. (Examples: *M. ovalis* A.S., *M. crucicula* (Grun) Cl., *M. binotata* (Grun) Cl.)

(2) Those forming a more or less complete ring, in which the compartments seem to be excavated. The loculi thus formed are sometimes arranged in layers, forming intricate patterns. (Examples: *M. fimbriata* (Brtw) Cl., *M. testudinea* Mfrd. Vgt., *M. Voigtii* Meist.)

(3) Those types in which the septate ring is composed of individual separate loculi, juxtaposed or nested, each unit being shaped like the head of a golf club (Fig. 14, 15), the shaft, often flattened or curved, being more or less developed. The analogy to the golf club is all the more striking as we find loculi of shapes corresponding to the different classes of golf clubs, with the characteristic width and thickness of the head and the angle of insertion of the shaft. When nested to form the ring, the heels are, in this type, placed on the outside, the shafts then generally lying in the direction of the apical axis of the valve, in the same plane as the loculi, which are thereby removed, more or less, from the margin of the valve. (Examples: *M. paradoxa* Grun., *M. imitatrix* A. Mann, *M. acuta* Grun., *M. lunula* Mfrd. Vgt.)

(4) Those forms which show the "iron" club shape of narrow loculi and inclined shafts, which lie in a second plane, above the septate ring, which they cross obliquely towards the outer margin of the valve. This rather complicated structure has the appearance of a filamentous membrane and has been described as such, but it can easily be shown, by mounting the specimens in thickened media, that both the loculi and the shafts are hollow and that their cavities communicate. In all cases, the entire arrangement of loculi and shafts is symmetrical to both main axes of the valve. (Examples: *M. decussata* Grun., *M. fallax* Cl., *M. viperina* Mfrd. Vgt.)

(5) Those species which apparently belong to the second type but in which only rudimentary shafts can be made out, the arrangement of the loculi showing the usual symmetry with regard to the axes; differences in the shape and size of the loculi often occur. (Examples: *M. robusta* Hust., *M. cruciata* (L.F.) Cl., *M. angulata* Lew., *M. Erythræa* Grun.)

It would seem that the third and fourth types represent the fundamental forms.

The loculi of the first two types often show minute pores, usually closed, by extremely thin membranes, which are more easily made out on specimens having suffered from the effects of excessive treatment by strong acids during the cleaning processes. This causes the outer layer or closing membrane to

curl and accentuates the appearance of the opening. (Examples: *M. splendidula* Hust., *M. emarginata* Hust.)

Figure 1005 of Hustedt's work, illustrating *Mastogloia mediterranea* Hust., shows minute spines on the inner margins of the loculi. These structures are, however, not confined to this one species but are found on a few other forms; being however extremely variable in development, they can hardly be utilized for purposes of identification. (Examples: *M. Grunowi* A.S., *M. Hustedti* Meister.)

Although, apart from these details, the walls of the loculi seem to be structureless, when examining isolated specimens of the large loculi found in *M. angulata* Lew., a very fine transverse striation, about 35–40 in 10μ, will be observed.

We know nothing about the functions of the loculi; Mann (1907) hints at their being of the same origin as the craticula often found in other naviculoid diatoms, but this is not the case, the craticula being an occasional result of variations in the living conditions of the cell, while the loculi are a constant feature of each species quite independent of outside conditions. The presence of pores would seem to indicate that the loculi are in some way connected with the nutritive processes or the regulation of the plasmic currents, but we have little information concerning Mastogloia in the living state and much work must still be done before any of these questions can be answered.

Without wishing to belittle the general accuracy and beauty of the numerous drawings accompanying Dr. Hustedt's publication, a word of warning seems indicated when they are to be used for purposes of identification in the absence of mounted specimens for comparison. Many of the objects portrayed are extremely minute and it is frequently necessary to exceed the useful magnification of the microscope in order to show and count their ultimate elements. The result is naturally a softening of contours which can only with difficulty be rendered by means of pen and ink sketches.

Much as this technique facilitates the work of the printer, a conflict inevitably arises, the draughtsman having to choose between showing lines as limits of adjacent rows of alveoli or puncta, or the imaginary centre-lines of the alveoli themselves. As the coarseness of the structure varies, a transition from one system to the other imposes itself and we frequently find both used. In some cases this renders rather difficult the correct interpretation of the drawing and the attempt to harness the natural artistry of the ornamentation of the cell, to a rigid system of crossed or parallel lines, defeats the very object for which it was designed, which was, to enable the student to identify and recognize the specimen.

Photographic reproduction of the microscopic image has its grave drawbacks and limitations, particularly in cases of extreme fineness of structure and lack of contrast or of objects situated at different focal levels, which should be shown in their correct relative positions, as is mostly the case with Mastogloia; but if executed with due care, a photomicrograph will usually not show structures which cannot also be made out and easily recognized by

ocular observation under the same conditions of illumination. The photograph will, on the other hand, mostly show those slight but characteristic half-tone and " shadow effects " which can only with the greatest difficulty and labour be rendered in drawings. A comparison of Hustedt's drawing No. 978, showing *M. cruciata* (L.F.) Cl., with Cleve's figure in the " Diatomiste " (1891) showing the same diatom and with the photograph Fig. 5, will make this clear.

The tropical and subtropical waters of the South China Sea, the East Indies and the islands connecting them with Southern Japan, are extremely rich in Mastogloia and the material used in these investigations was mostly collected in these regions as well as along the steamer routes between Europe and the Far East; part of it is also derived from Trepang from the Dutch East Indies, especially Celebes, and from Malaya.

In examining this material, many forms were encountered which could not be identified with any published species and which may therefore be considered as new, while it was also possible definitely to establish the identity and habitat of some doubtful species and to extend the data concerning the dimensions and structure of others. The results of this work have been tabulated alphabetically in the following paragraphs.

(1) *Mastogloia acutiuscula* Hust. (Hust., p. 515, fig. 947.)

Was found in the harbour of Djibouti, among filamentous algæ.

(2) *Mastogloia admirabilis* n.sp., fig. 37.

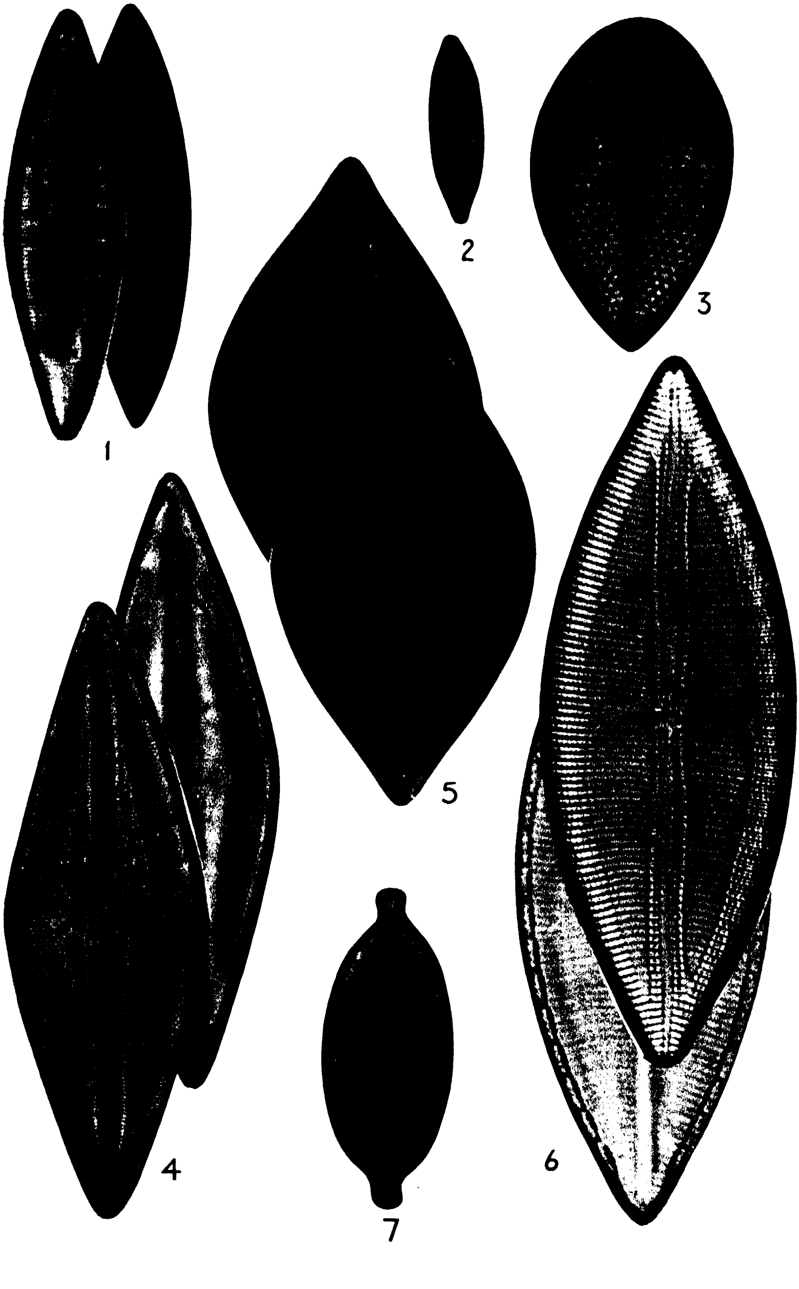
Valvis elliptico-lanceolatis 60–87μ longis, 22–7μ latis, apicibus obtusis, subproductis; area centralis rotundata, axillaris angusta inter sulcas parallelas ad centrum non interruptas; raphe undata; striis transversis leniter radiantibus 17–18 in 10μ, punctatis, puncta in 20–22 lineas rectas longitudinalis in 10μ ordinatis; loculamentis m. citrus similibus, 12–13 in 10μ, 4·5μ latis, ad apices attingentibus. In aquis marinis ad insulam Celebes.

This rare species belongs to Hustedt's " apiculatæ " group and combines the striation of *M. apiculata* with the loculi structure of *M. citrus*. It differs from the former in having an undulate raphe and a small though well defined central area. The loculi are both deeper and narrower than those of *M. apiculata*, reaching well into the apices; in these respects it resembles *M. citrus*, which is common in the same material; no intermediate forms were, however, found, connecting the two species.

(3) *Mastogloia amoyensis* n.sp. Fig. 1.

Lanceolata, 33–37μ longa, 8–9·6μ lata, apicibus acutis; area centralis parva, axilaris angusta; striæ transversæ parallelæ, punctatæ, 25–30 in 10μ, punctis elongatis in lineas longitudinales undulatas ordinatis; loculamenti m. pumilæ similis. In aquis marinis ad Amoy et Belawan.

This small form resembles *M. pumila*, but is more rhombic in outline,



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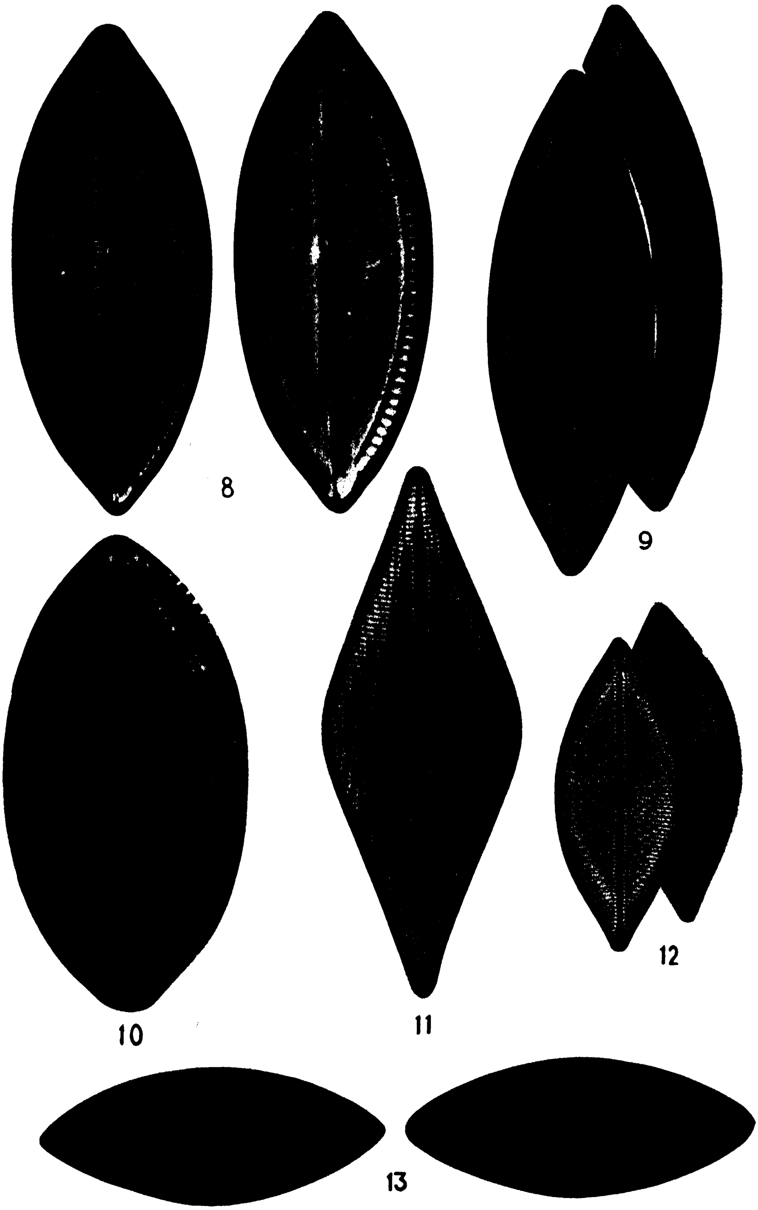
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without the subrostrate apices of the latter. The punctuation, though very faint, is distinct. The hyaline H-shaped mark, characteristic of *M. pumila*, is also wanting.

(4) *Mastogloia amphipleuroidea* n.sp. Fig. 40.

Valvis lanceolatis apicibus acutis 26–40μ longis, 6–8μ latis ; raphe undata, poris celtralibus approximatis ; area axillaris angusta, centralis parva ; striis transversis 25–30 in 10μ, parallelis ; loculamentis 6 in 10μ, ad apices attingentibus, 1μ latis, marginibus convexis. In aquis marinis ad insulam Celebes.

This is a small diatom which slightly resembles *Amphipleura pellucida* in outline and striation, while the loculi are of the *M. angusta* type. The striation is much finer than that of the latter species, and, when examined under oblique illumination, it will be found that the striæ half way between the centre and the apices are very slightly radial, the remainder being parallel.

(5) *Mastogloia angulata* Lewis. (Hust., p. 465, fig. 885.)

This is very common in the East Indies, specimens found in Holothurians from Celebes exceeding the maximum dimensions given by Hustedt. Length up to 93μ, breadth 29μ, central loculi 5, 5μ deep, 1, 5 in 10μ, lateral loculi 2–3μ deep, 3 in 10μ.

(6) *Mastogloia apiculata* W. Sm. (Hust., p. 515, fig. 946.)

This species, characterized by the straight raphe and parallel, uninterrupted straight ribs which enclose it, is also found in the East Indies and in Djibouti, as well as along the China Coast. The density of striation was found to vary between 16 and 19, 5 in 10μ, while Hustedt gives it as about 20.

(7) *Mastogloia aspera* (Hust.) n.nom. (Hust., p. 480, fig. 901.) Fig. 31.

In the note accompanying his diagnosis, the author draws attention to the uncertainty in the identification of Grunow's *M. asperula*. He shows the alveoli as hexagonal and arranged in almost parallel transverse rows, while Cleve's description and figure of the same species in the " Diatomiste " (1891) gives the alveoli as elongated and arranged in radial lines.

Confident in the existence of only two distinct species, Hustedt has grouped all the larger and coarsely marked forms under the species *M. asperula*, regardless of Cleve's description of the alveoli, and has formed a new species, *M. asperuloides*, comprising the smaller finely marked forms, with elongated alveoli.

Examination of a large amount of material from Celebes disclosed the fact that not two but three similar, but quite distinct species occur there, one of which, with elongated, tear-shaped alveoli, is found to coincide almost exactly in all its characteristics, with Cleve's description and figure, and can

therefore be taken to represent the true *M. asperula* Grun. Hustedt's form, with more circular or hexagonal alveoli, which is also common in this material, has therefore been renamed *M. aspera*. It is easily distinguished by its less radial striæ and by the secondary structure of the alveoli, which is shown in Hustedt's figure.

(8) *Mastogloia asperula* Grun. (Cleve Diatomiste I, p. 161, Pl. 23, f. 12.) Fig. 32.

Although Cleve states in his diagnosis that the striæ are slightly radial, his figure shows them very strongly so, especially in the central portion of the valve; this feature also characterizes the Celebes specimens. The inner margins of the loculi are straight and no secondary structure is visible in the alveoli.

(9) *Mastogloia asperuloides* Hust. (Hust., p. 482, fig. 902.)

This is common in Nagasaki, the Loochoo Islands and Chusan Island. The dimensions correspond to the diagnosis given, but the striation has been found to be coarser, 17–18 in 10μ.

(10) *Mastogloia bahamensis* Cl. (Hust., p. 530, fig. 963.)

Specimens from N. Sumatra, Singapore, and Celebes, show dimensions exceeding so considerably the limits set by Hustedt, that they might almost be classified as a separate variety, the form being also more rhomboidal; however, intermediate forms occur. Length 80–138μ, breadth 28–35μ, striæ 9·5–13 in 10μ.

(11) *Mastogloia balteata* n.sp. Fig. 2.

Valvis ellipticis 15–16μ longis, 4–5μ latis, apicibus productis, obtusis; raphe directa; striis tenuissimis, transversis, parallelis, 32 in 10μ, ad medium valvæ admodum separatis; loculamenti utrimque unus. Ad Nagasaki et Insulas Loochoo, in aquis marinis.

This minute form is easily recognised by the belt, formed by the coarser median striæ; no puncta can be made out in Hyrax mounts.

(12) *Mastogloia binotata* (Grun.) Cl., f. ovata n.f. Fig. 3.

The type is very common; it occurs in Nagasaki and in Djibouti in a distinctly ovate form.

(13) *Mastogloia bipartita* n.sp. Fig. 36.

Valvis ellipticis 40–45μ longis, 12–13μ latis, apicibus obtusis, rotundatis; raphe directa; area axillaris angustissima, incisa, centralis parva; striis transversis parallelis, 25 in 10μ, inconspicue punctatis; loculamentis 7 in 10μ, 22, 5μ latis, marginibus rectis, ad apices attingentibus. Ex holothuriis Celebes insulæ.

This rare species is characterized mainly by the deeply cut linear axial area, which seems to bisect the valve. The striation, parallel throughout,

is rendered slightly asymmetrical by the insertion of one or two shortened striæ on one side of the central area. The puncta are extremely fine and can only with difficulty be resolved in Hyrax mounts.

(14) *Mastogloia cebuensis* A. Mann. (1925). Fig. 4.

Diatoms which seem to correspond to Mann's figure are not uncommon in Nagasaki and Singapore : outline rhombic, length 110–155μ, breadth 35–48μ, striæ slightly radial, 11–14 in 10μ, puncta in the outer zone 9–11 in 10μ, arranged in irregular oblique lines. The inner portion of the valve surface, also rhombic in outline, is covered with faint and interrupted continuations of the transverse striæ and widely spaced, scattered puncta, forming wavy longitudinal lines ; two or three more regular lines of puncta accompany the axial area. Central area large, rounded and expanded outwards ; raphe slightly bent ; loculi reaching into the apices, 1μ deep, 2–3 in 10μ with convex inner margins.

There seems to be considerable confusion concerning the delimitation of the species *Mastogloia cebuensis*, *M. lemniscata* and *M. Jelinecki*. Mann considers his specimen of *M. cebuensis* to be identical with Cleve's Fig. 26, Pl. II, of the "Naviculoid Diatoms" (1894), there named *M. lemniscata.* Mann's figure is not very satisfactory, owing probably to the fact that he attempted simultaneously to focus the surface marking and the loculi ; it shows however that the structure of the loculi differs very considerably from the deep rectangular form shown both in Cleve's work (1895) and in Hustedt's figure 976 of *M. lemniscata.* It more nearly corresponds to that of *M. strigilis* Hust. or *M. Jelinecki* Grun., so that Hustedt's remark (p. 544) that *M. cebuensis* is synonymous with *M. lemniscata*, is hardly admissible.

The length, according to Mann, is 125μ, thus exceeding the maximum value given for *M. lemniscata* and for *M. Jelinecki*, while the specimens found in Singapore and which seem to be identical with Mann's *M. cebuensis*, attain a length of 155μ. It would seem therefore that *M. cebuensis* should be retained as a separate species.

(15) *Mastogloia celebensis* n.sp. Fig. 34.

Valvis lanceolatis apicibus acutis, 40–53μ longis, 12–14μ latis ; striis transversis radiantibus 19 in 10μ, punctatis, punctis 21–22 lineas longitudinales in 10μ leniter undulatas constituentibus. Raphe recta, inter lineas parallelas ad centrum interruptas et devergentes. Area axillaris angusta, centralis rotundata ; loculamentis 2μ latis, 5 in 10μ, ad apices attingentibus, marginibus rectis. In aquis marinis ad insulam Celebes.

This form was found to be remarkably constant in proportions and striation and cannot well be classed with either *M. labuensis*, *M. robusta*, or *M. acutiuscula.* It differs from the first, in the comparatively large, rather irregularly defined central area, the total interruption of the ribs enclosing the axial area, which flare at the central area, as well as the straight inner margins of the loculi, which reach into the apices. It is much more slender

in outline than the typical *M. robusta*, with more acute apices and a straight raphe. This feature might connect it with *M. apiculata*, were it not for the larger central area and the interrupted longitudinal ribs.

(16) *Mastogloia citrus* Cl. (Hust., p. 519, fig. 954.)

This species is widely distributed in the East Indies and along the China Coast, the Northern limit being probably Wei Hai Wei, where a few specimens were found on the sands, at low tide.

(17) *Mastogloia consticta* Cl. (Hust., p. 506, fig. 931B.)

Material from Celebes contains a very much more constricted form of this species, the loculi almost touching in the waist. Length 35–40μ, breadth at the broadest part, 6–7μ and 4μ at the waist.

(18) *Mastogloia cruciata* (L–F.) Cl. (Hust., p. 546, fig. 978.) Fig. 5.

The accompanying photographs were made from material collected at Colombo and agree very well with the original figure given by Cleve (1891), while Hustedt's drawing does not indicate the uninterrupted transverse striation covering the central portion of the valve, with the exception of the narrow hyaline stauros which gave the species its name. The puncta in the outer zone are arranged in very irregular oblique secondary lines. While the overall dimensions agree with the data of both publications, the density of striation was found not to exceed 9–10 in 10μ, which here, again, is in agreement with Cleve's indications ; Hustedt gives it as 12–13.

(19) *Mastogloia cruciata v. elliptica* n.v. Fig. 6.

The variety differs from the type in its more elliptico-lanceolate outline, with subrostrate apices, its greater size and even coarser striation. Length 76–158μ, breadth 35–54μ, striæ 7–9 in 10μ, loculi 2 in 10μ, 2·5μ deep with convex inner margins. It is common in Celebes, Hainan Island and the West coast of Malaya.

(20) *Mastogloia cyclops* n.sp. Fig. 8.

Valvis elliptico-lanceolatis, apicibus productis, 30–45μ longis, 16–22μ latis ; striis punctatis transversis, 21–22 in 10μ, punctis lineas longitudinales leniter arcuatas constituentibus ; cum ocello magno unilaterale. Loculamentis 10–12 in 10μ, 2μ latis, ad apices attingentibus ; raphe ad centrum inflexa. In aquis marinis ad Nagasaki et Indias Orientales.

The circular oculi, similar to those found in some species of Cocconeis, are on opposite sides of the apical axis, one on each valve. The inner margins of the loculi are slightly convex.

(21) *Mastogloia decipiens* Hust. (Hust., p. 504, fig. 929.)

This species also occurs in Singapore.

(22) *Mastogloia decussata* Grun. (Hust., p. 493, fig. 917.)

The typical *M. decussata* is lanceolate in outline, with very slightly subrostrate apices; it occurs in Celebes, in an elliptical lanceolate form, with acute cuneate apices. Characteristic for both is the very broad belt, composed of narrow loculi, showing well-developed shafts.

(23) *Mastogloia delicatissima* Hust. (Hust., p. 482, fig. 903.)

Specimens corresponding to the diagnosis, but slightly larger, were found in Nagasaki. Length up to 27μ, breadth 11μ.

(24) *Mastogloia dicephala* n.sp. Fig. 7.

Valvis ellipticis, apicibus capitatis, 23–33μ longis, 10–12·5μ latis; striis punctatis radiantibus, 22–23 in 10μ, punctis elongatis in lineas rectas longitudinales ordinatis; area axillaris et centralis angustæ; loculamentis utrimque 2–5, 1·5–2μ latis, 3–4 in 10μ, marginibus convexis. In aquis marinis ad Singapore et Celebes.

This is a form resembling *M. decipiens* Hust., but is more coarsely marked, with smaller, capitate apices and more convex and larger loculi; the longitudinal lines are very faint.

(25) *Mastogloia dissimilis* Hust. (Hust., p. 492, fig. 916.)

This is common in Wei Hai Wei, Singapore and Celebes, where it occurs together with *M. decussata*, which it resembles, except for the narrower loculi and the smaller dimensions.

(26) *Mastogloia divergens* A.S. (Hust., p. 572.) Fig. 9.

This species is noted as doubtful, both by Hustedt and Cleve. It is shown in Fig. 52 of Plate 187 of Schmidt's Atlas, but no description seems to have been published, so that a diagnosis of specimens obtained in Djibouti may be of interest.

Form, elliptico-lanceolate with rostrate apices, length 54–85μ, breadth 22·5–23, 5μ, surface strongly convex; transverse striæ parallel, 16, 5 in 10μ, composed of circular puncta arranged in irregular wavy longitudinal lines, appearing decussate towards the margin of the valve, owing to the convexity of the surface. Raphe straight, central pores approximate and terminal fissures small, hooked. Axial area narrow, linear, central area small, irregular in outline and slightly dilated by the shortening of one or two striæ.

The raphe is situated on a spindle-shaped elevated area, bordered on either side by depressions which are shallow in the centre and more marked towards the apices; in each quadrant thus formed, there is a pair of sharply defined, parallel furrows, diverging in the middle of the valve, where they terminate in the already mentioned depressions, the other ends not reaching the apices. The loculi, 8 in 10μ, are very narrow and difficult to make out, owing to the convexity of the valve.

(27) *Mastogloia (?) dubitabilis* Meister (1937). Fig. 10.

This curious species occurs in isolated specimens along the China coast as far North as Tsingtao and Wei Hai Wei. Attempts to isolate the apparent loculi from the valve have failed and it seems doubtful whether it can be regarded as a true *Mastogloia.* It is perhaps identical with Greville's *Navicula luxuriosa* (1863).

(28) *Mastogloia elegantula* Hust. (Hust., p. 541, fig. 975.) Fig. 11.

A rare and beautiful diatom, also found in Celebes; length 33–65μ, breadth 18–20μ (Hustedt's figure, 10μ is evidently a printer's error as it does not agree with his drawing), striæ 18 in 10μ, loculi 3 in 10μ, 1μ deep.

(29) *Mastogloia emarginata* Hust. (Hust., p. 477, fig. 896.)

So far only found in Borneo, this species also occurs in the Chusan Islands.

(30) *Mastogloia Erythræa* Grun. (Hust., p. 524, fig. 959.)

All specimens collected in Djibouti and in the China Seas, show two short ribs along the outer part of the axial area, which are indicated in Fig. 37, Plate 186 of Schmidt's Atlas, but do not figure in Hustedt's drawing.

(31) *Mastogloia fallax* Cl. (Hust., p. 504, fig. 930.)

Diatoms which can only be assigned to this species were found in Singapore and Amoy; the dimensions, however, greatly exceed the limits set by Hustedt and the marking is coarser. Length 40–71μ, breadth 15–19·5μ, striæ 21–23 in 10μ.

(32) *Mastogloia Frickei* Hust. (Hust., p. 534, fig. 967.)

A very curiously marked diatom which occurs also in Celebes; the border of the inner striated zone seems generally less sharply defined than shown in Hustedt's drawing. Length 40–51μ, breadth 12–13μ.

(33) *Mastogloia fusiformis* A. Mann (1925). Fig. 12.

Hustedt considers Mann's specimen to have been a coarsely marked *M. Jelinecki.* This is difficult to judge without having access to the original material, but specimens collected in Nagasaki and on the West coast of Malaya show plainly the "brick wall" pattern mentioned by Mann and without the central cross ridge which is characteristic of *M. Jelinecki*; so that the retention of *M. fusiformis* as a separate species seems justified. Length 70μ, breadth 32μ, striæ 10 in 10μ, loculi 4 in 10μ, 1·2μ deep.

(34) *Mastogloia gracilis* Hust. (Hust., p. 507, fig. 933.)

Some very beautiful examples of this species were found in the Celebes material; length up to 63μ, breadth to 18μ, striæ 16–17 in 10μ.

(35) *Mastogloia graciloides* Hust. (Hust., p. 508, fig. 934.)

This was found in Celebes and Singapore, where it is rather larger and coarser marked and shows a clearly defined central area. Length 40μ, breadth $14{\cdot}5\mu$, striæ 18 in 10μ, loculi 7–8 in 10μ, 2μ deep, the terminal loculi greatly lengthened.

(36) *Mastogloia Grunowi* A.S. (Hust., p. 555, fig. 988.)

This is very common in the whole of the Far East, especially in the Chusan Islands, where most specimens show well-developed spines on the inner margins of the loculi.

(37) *Mastogloia Horvathiana* Grun. (Hust., p. 471, fig. 890).

Cleve (1895) gives the length of this species as 35μ, and breadth 24μ; Hustedt indicates 30–60μ and 22–43μ. Very large, elongated specimens occur in the Celebes material, necessitating a further extension to 30–83μ for the length and 22–43μ for the breadth, the transverse striation remaining 11 in 10μ, and the loculi 6–8 in 10μ, 4–$4{\cdot}5\mu$ deep.

(38) *Mastogloia Hustedti* Meister (1935). Fig. 13.

The loculi of this species also commonly bear spines and the surface marking is very similar to that of *M. Grunowi,* except for the well-defined rectangular central area. The main difference, however, which is not well shown in Meister's figure, is to be found in the shape of the loculi, which are stepped in width, towards the apices, while those of *M. Grunowi* are gradually reduced. It occurs in Bali, Singapore, the Chusan Islands and the West coast of Malaya.

(39) *Mastogloia imitatrix* A. Mann (1925). Fig. 14.

The species is listed as doubtful by Hustedt, but specimens were found in Amoy and Singapore which correspond closely to Mann's description. It will be seen that the arrangement of the terminal loculi differs somewhat from that rather sketchily shown in Mann's figure, but this can be explained by the movement of the specimen during the execution of the drawing. *M. imitatrix* gives us a most typical example of loculi type 3. (Fig. 15.)

(40) *Mastogloia impressa* Hust. (Hust., p. 555, fig. 987.)

This is a rare species found by the author in material from Funafuti; it also occurs in Nagasaki and the Loochoo Islands.

(41) *Mastogloia javanica* Cl. (Hust., p. 589, fig. 973.)

Found also in Singapore and very common in Celebes. Length 90–125μ, breadth 30–7μ, striæ 13 in 10μ, loculi corresponding to fig. 38, pl. 188 of Schmidt's Atlas, rather than to Hustedt's figure.

(42) *Mastogloia Jelinecki* Grun. (Hust., p. 544, fig. 977).

Hustedt draws attention to the difficulty of ascribing limits to this very variable species. One of the essential features would seem to be the presence of the elevated transverse ridges, which constitute one of the differences between this species and *M. Cebuensis.*

(43) *Mastogloia Kjellmani* Cl. (Hust., p. 491, fig. 915).

Occurs in Singapore.

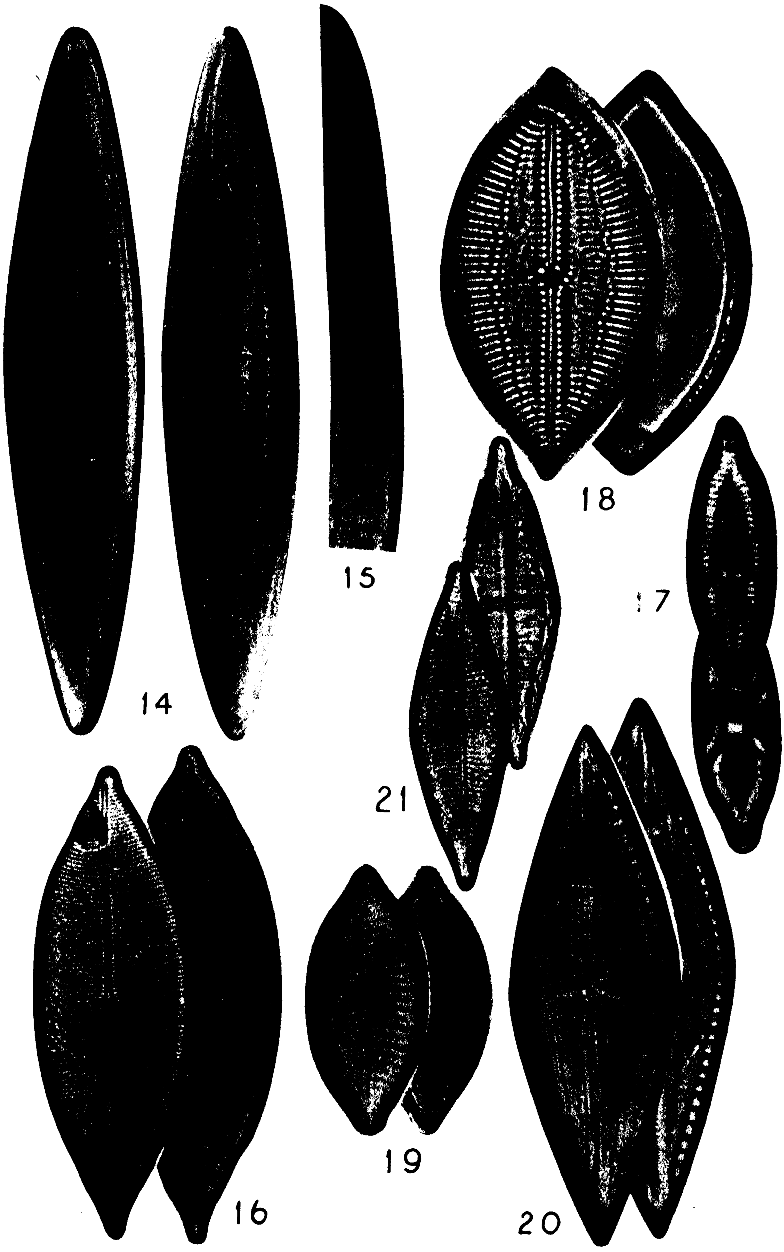
(44) *Mastogloia labuensis* Cl. (Hust., p. 518, fig. 950).

M. acutiuscula, M. labuensis, and *M. robusta,* together with their varieties, resemble each other sufficiently to make their separation rather difficult. Hustedt's identification of *M. labuensis* is based upon examination of original specimens from Labuan and should therefore give us the correct specification, except that he gives the density of striation as 20–22 in 10μ, against Cleve's counts of 15–17. On comparing the drawings published by the two authors, one is struck by the fact that the outline of all of Hustedt's figures is more or less elliptical, though in the text he says "linear with parallel sides," while Cleve's figure is actually linear. This author states that the raphe is straight, while Hustedt, though showing it straight or only slightly bent, mentions in the text that it is bent. The loculi, according to Hustedt's figures and text, have a convex margin, while Cleve's figure shows them straight; it should, however, be remembered that little attention was paid at the time of Cleve's publication to this important feature.

The specimens found in the Celebes material agree in general with Hustedt's description, the sides being more or less convex, but the striation varies from 16 to 21 in 10μ. The same material contains, however, a great number of forms with parallel or even slightly concave sides and obtuse cuneate apices, with strongly sinuous raphe, more or less radial striæ, 15–17 in 10μ, and loculi with straight inner margins and plainly visible shafts. With the exception of the sinuosity of the raphe, which might easily have been overlooked by Cleve, these correspond fairly well to his figure; similar types have been found in N. Sumatra, Singapore, Bali, and the Chusan Islands.

Assuming that Hustedt's figures, drawn according to the original Labuan material, can be relied upon, then the true *M. labuensis* would be characterized by more or less convex sides, obtuse cuneate apices, straight or only slightly bent raphe, between continuous parallel longitudinal ribs and loculi with convex inner margins; length up to 92μ, breadth 10–16μ, almost parallel longitudinal and transverse striæ 16–22 in 10μ and narrow axial and central areas.

The coarsely marked forms, with strongly sinuous raphe, radial transverse striæ and loculi with straight inner margin, must then be considered as a variety of *Mastogloia robusta* Hust., and it seems probable that the presence



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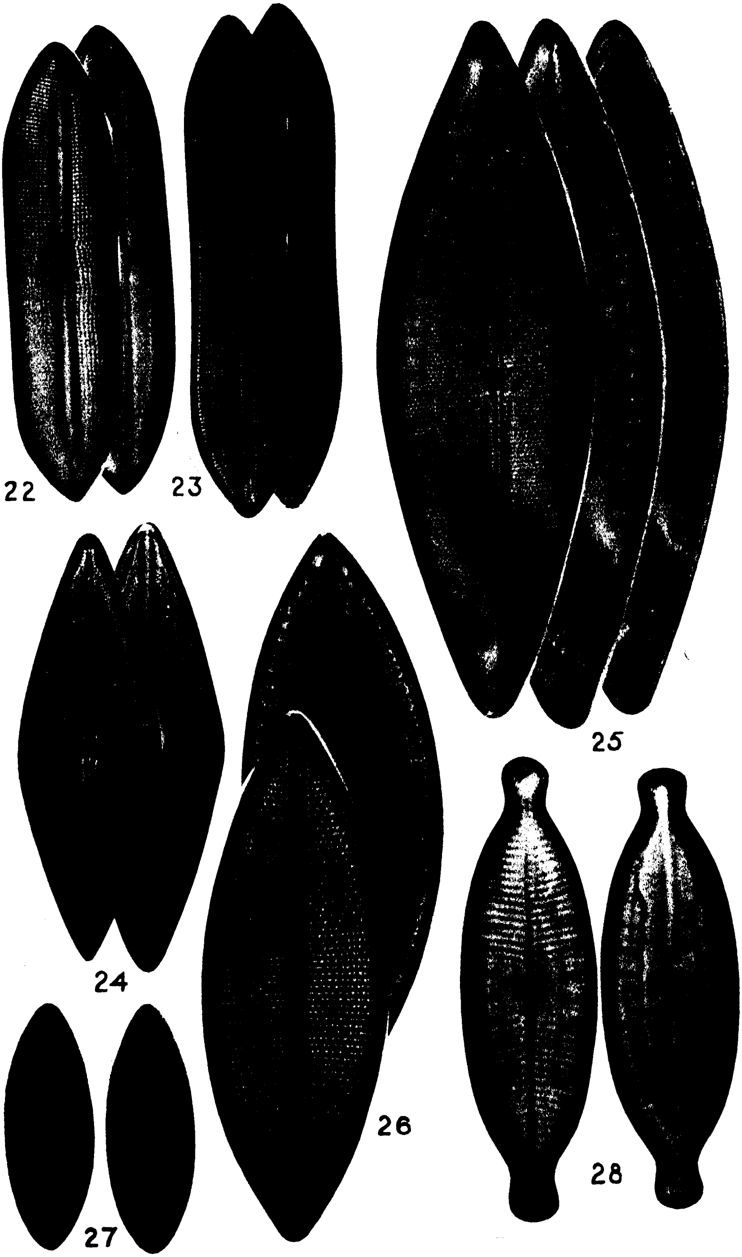
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in his slides of some of these specimens was responsible for the figure published by Cleve, who did not differentiate the species.

(45) *Mastogloia latericia* (A.S.) Cl. (Hust., p. 480.)

This rare species was found in Holothurians from the Dutch East Indies (probably Celebes). Length 46μ, breadth 20μ, striæ 11 in 10μ, alveoli 7 in 10μ, loculi $2{\cdot}5\mu$ deep, 4 in 10μ, with concave inner margins.

(46) *Mastogloia lemniscata* L–F. (Hust., p. 543, fig. 976.)

Hustedt's text mentions the faint prolongations of the striæ which cross the inner zone of the valve. The species is easily recognised by these, in combination with the scattered longitudinal lines of puncta in the inner zone and the arrangement of the puncta of the outer zone in lines roughly parallel to the margin of the valve.

(47) *Mastogloia lentiformis* n.sp. Fig. 33.

Valvis latis, elliptico-lanceolatis, apicibus acutis, 8–10μ longis, 6–7μ latis; striis radiantibus, arcuatis 32 in 10μ, ad centrum densioribus; raphe directa cum poris centralibus approximatis; loculamentis centralibus magnis, a latere minoribus. In aquis marinis ad Celebes.

A minute form, with strongly convex surface. No puncta can be made out in Hyrax mounts.

(48) *Mastogloia lunula* n.sp. Fig. 39.

Valvis elliptico-lanceolatis 40–73μ longis, 13–14, 5μ latis, apicibus productis, obtusis; striis transversis et longitudinalis rectis, parallelis, 27 in 10μ; raphe sinuosa inter lineas parallelas ad centrum interruptas; poris centralibus approximatis. Area axillaris angusta, centralis parva; loculamentis in medianas breves lineas arcuatas dispositis, 4 in 10μ, medio 2μ latis, hinc ad apices leniter decrescentibus. In aquis marinis ad Celebes.

This form might easily be mistaken for *M. seychellensis* Hust., were it not for its strongly sinuous raphe and the enclosing longitudinal ribs, which are wanting in the latter. *M. seychellensis* seems not to occur in Celebes.

(49) *Mastogloia mammosa* n.sp. Fig. 16.

Valvis elliptico-lanceolatis polis productis, mammiformis, 64–75μ longis, 20–22μ latis; striis leniter radiantibus ad apices arcuatis 17–17·5, in 10μ; punctis elongatis 8 in 10μ, lineas undulatas obliquas constituentibus; area axillaris angusta, centralis parva, paulum dilatata; loculamentis angustissimis, 10 in 10μ, propter superficiem convexam valvæ difficulter visibilis. Ex holothuriis Indiarum Orientalium.

This diatom resembles slightly Cleve's *M. dubia*, which is a fossil species from Barbadoes; as in the case of *M. divergens*, the loculi are very difficult to make out and can best be seen on broken valves.

(50) *Mastogloia mauritiana* Brun. (Hust., p. 563, fig. 995.)

Hustedt shows only the elliptico-lanceolate subrostrate type of this species which is, however, extremely variable in size and outline. In addition to the localities enumerated, it occurs in Nagasaki, the Chusan Islands, Bali and Celebes. In the last-named locality we find very large specimens of rhombic lanceolate outline as shown in Fig. 4, Plate 6 of Peragallo's "Diatomées maritimes de France," where it is misnamed *M. quinquecostata v. Hantzschii* Grun. The same material contains elliptico-lanceolate types attaining a length of 87μ and a breadth of 24μ, with 19–21 striæ in 10μ, while a small elliptical type is only 17μ long and 10μ wide, with 24 striæ in 10μ. (Fig. 27.) All sorts of intermediate forms occur, so that it is impossible to separate well-defined varieties.

(51) *Mastogloia mauritiana v. capitata* n.v. Fig. 35.

The same cannot be said of this form, with rostrate to capitate apices and broad elliptical valves, straight to slightly sinuous raphe and fine radial and curved transverse striæ. The longitudinal slightly convex depressions which characterize the species, are often very faint in the variety, while the puncta are so fine that they can hardly be made out in Hyrax mounts. The loculi are of the form common to the species and reach well into the apices. Length 35–37μ, breadth 17–19μ, striæ 20–22 in 10μ, depth of the loculi 1·5 and 2·5μ respectively. Rare, in Celebes.

(52) *Mastogloia minutissima* n.sp. Fig. 17.

Valvis ellipticis, polis obtusis rostratis, 16·5μ longis, 5·5μ latis; raphe directa, area axillaris angusta, ad centrum leniter dilatata, centralis parva, elliptica, ad forman lyræ dilatata; striæ transversæ ad polos radiantes, 28–30 in 10μ; loculamenti utrimque unus, 2μ latus, semirotundus. Ex holothuriis Indiarum Orientalium.

This is a very small form, similar to *M. mediterranea* Hust. in surface marking but differing therefrom in the shape of the valve and number and arrangement of the loculi. It is perhaps identical with *M. capitata* Brun. (Peragallo, 1897).

(53) *Mastogloia modesta* n.sp. Fig. 30.

Valvis linearibus apicibus cuneatis subproductis, 50–68μ longis, 15–18μ latis; striis transversis 16–17 in 10μ, ad apices subradiantibus, punctatis; punctis elongatis, 7–8 in 10μ, in lineas longitudinales undulatas ordinatis. Area axillaris angusta, centralis parva; loculamenti angustissimi, 6–10 in 10μ, ad apices attingentes. In aquis marinis ad Celebes.

This is another species in which the convexity of the valve and the heavy silicification render the observation of the loculi very difficult.

(54) *Mastogloia obesa* Cl. (Hust., p. 548.) Fig. 18.

Hustedt has not found this species and gives no figure of it ; the original habitat is Java, but it also occurs in Singapore and on the West coast of Malaya, on oyster shells.

(55) *Mastogloia orientalis* n.sp. Fig. 19.

Valvis ellipticis, polis subrostratis, obtusis, 21μ longis, 9μ latis ; area axillaris angusta, centralis parva, rotundata ; striis radiantibus 14–15 in 10μ, punctatis, puncta lineas longitudinales arcuatas, 21 in 10μ constituentes ; raphe leniter undata ; loculamentis 8 in 10μ, 1·5–2μ latis, ante polos interruptis. In aquis marinis ad Nagasaki.

The valves of this rare species are comparatively coarsely marked and heavily silicified.

(56) *Mastogloia panduriformis* n.sp. Fig. 38.

Valvis panduriformis, apicibus rostratis, 50–55μ longis, 9 et 13μ latris ; striis radiantibus 15 in 10μ crasse punctatis ; area axillaris lanceolata, centralis rotundata ; raphe undata ; loculamentis 5 in 10μ, 1–1·5μ latis ad apices attingentibus. Ex holothuriis Celebes insulæ.

This differs from *M. constricta* in form, striation and shape of the areas ; both species occur in the Celebes material, without intermediate forms.

(57) *Mastogloia peracuta* Jan. (Hust., p. 485, fig. 906.)

This species, previously known only from the " Gazelle " soundings, was found in the Chusan Islands and in material from a cable sounding off Nagasaki.

(58) *Mastogloia pisciculus* Cl. (Hust., p. 558, fig. 990.)

Occurs in Nagasaki and is very common in Celebes.

(59) *Mastogloia pseudocruciata* n.sp. Fig. 20.

Valvis rhombeo-lanceolatis, polis acutis, 70–87μ longis, 24–27μ latis ; area axillaris angusta, centralis stauriforme dilatata ; raphe undata ; striæ radiantes, 18–20 in 10μ, marginales punctatæ, punctis in lineas irregulares, ad raphem in lineam directam dispositis ; zona centralis valvæ leviter striata ; loculamenti rectangularis 4–4·5 in 10μ, 2–3μ lati ad polos attingentes. Ex holothuriis Indiarum Orientalium.

Two specimens only of this rather doubtful species were found. It resembles *M. lemniscata*, except for the structure of the lateral areas and the presence of the narrow hyaline stauros, which would connect it with *M. Macdonaldi* or *M. foliolum* ; it differs from the latter two in form, dimensions, the sinuous raphe, and the shape and arrangement of the loculi, which are all similar in size and show straight inner margins.

(60) *Mastogloia pseudoparadoxa* Hust. (Hust., p. 521, fig. 954.)

This is common in Sabang, N. Sumatra, where it attains a length of 98μ and a width of 24·5μ.

(61) *Mastogloia quinquecostata* Grun. (Hust., p. 556, fig. 989.)

The extreme variability of this species renders identification uncertain; it is very common in the Far East. Hustedt's data must be slightly extended: length 30–125μ, breadth 14–37·5μ, striæ 15–20 in 10μ; specimens from Celebes and Wei Hai Wei show a more or less rhombic outline with undulate margins, but a separation of these forms is not justified, as intermediate forms between these and the true elliptico-lanceolate types occur.

(62) *Mastogloia robusta* Hust. (Hust., p. 518, fig. 981.)

This species has already been mentioned under *M. labuensis*; it differs from the latter in its radial striæ, sharply defined, rounded central area, with corresponding curvature of the longitudinal ribs enclosing the raphe, sinuous raphe and the longitudinal striæ, which are more widely spaced than the transverse ones. The loculi have a flat inner margin and plainly show the presence of the superimposed rudimentary shafts. The striation is very variable but usually coarser than that of *M. labuensis.*

(63) *Mastogloia robusta v. subconstricta* n.v. Figs. 22, 23.

Many specimens, which, as mentioned under *M. labuensis,* otherwise correspond to the diagnosis of *M. robusta,* are linear or even slightly constricted, with obtuse cuneate apices; length 64–87μ, breadth 17–18μ, striæ 16 in 10μ, puncta 13–15 in 10μ; loculi 7–8 in 10μ, 2–3μ deep. The longitudinal ribs are sometimes more or less interrupted at the central area.

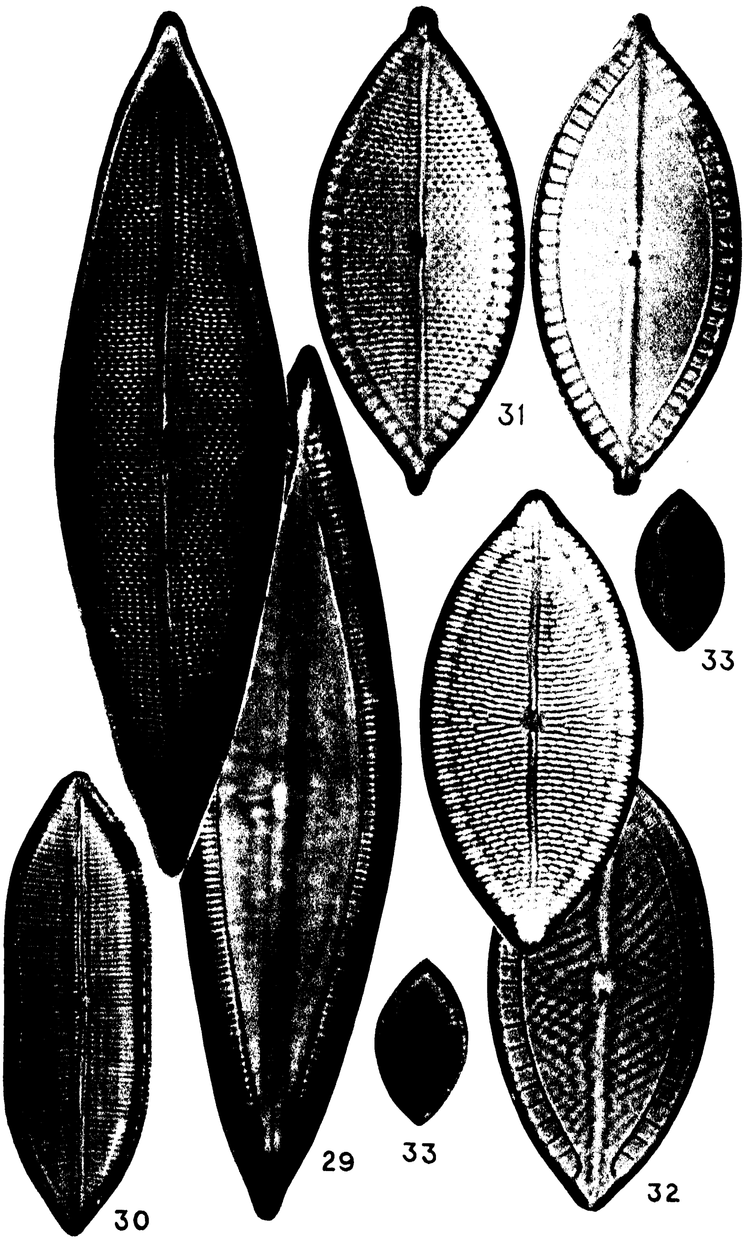
(64) *Mastogloia rugosa* n.sp. Fig. 24.

Valvis rhombeo-lanceolatis apicibus obtusis, 38–68μ longis, 12·5–23μ latis; striis marginalibus radiantibus, punctatis, 17–21 in 10μ; punctis in lineas irregulares longitudinales ordinatis; raphe leniter inflexa, in sulco lineis punctorum elongatorum cincta; area axillaris angusta, centralis parva, quadrata; utrimque area nuda, semilunaris, punctis obscuris et irregularis signata. Loculamentis angustissimis, 1–1·5μ latis, 2·5–3 in 10μ, convexis, ad polos attingentibus. In aquis marinis ad insulas Celebes et Singapore.

The marking of this diatom resembles slightly that of *M. lemniscata,* the lateral areas are, however, granulated, not interrupted by a stauroid central area and without any signs of transverse lines; the loculi are also totally different, resembling more those of *M. Jelinecki.*

(65) *Mastogloia serrata* n.sp. Fig. 21.

Valvis lanceolatis, polis productis, 39·5μ longis, 12μ latis; raphe sinuosa; area axillaris angustata, centralis ad formam stauri dilatata



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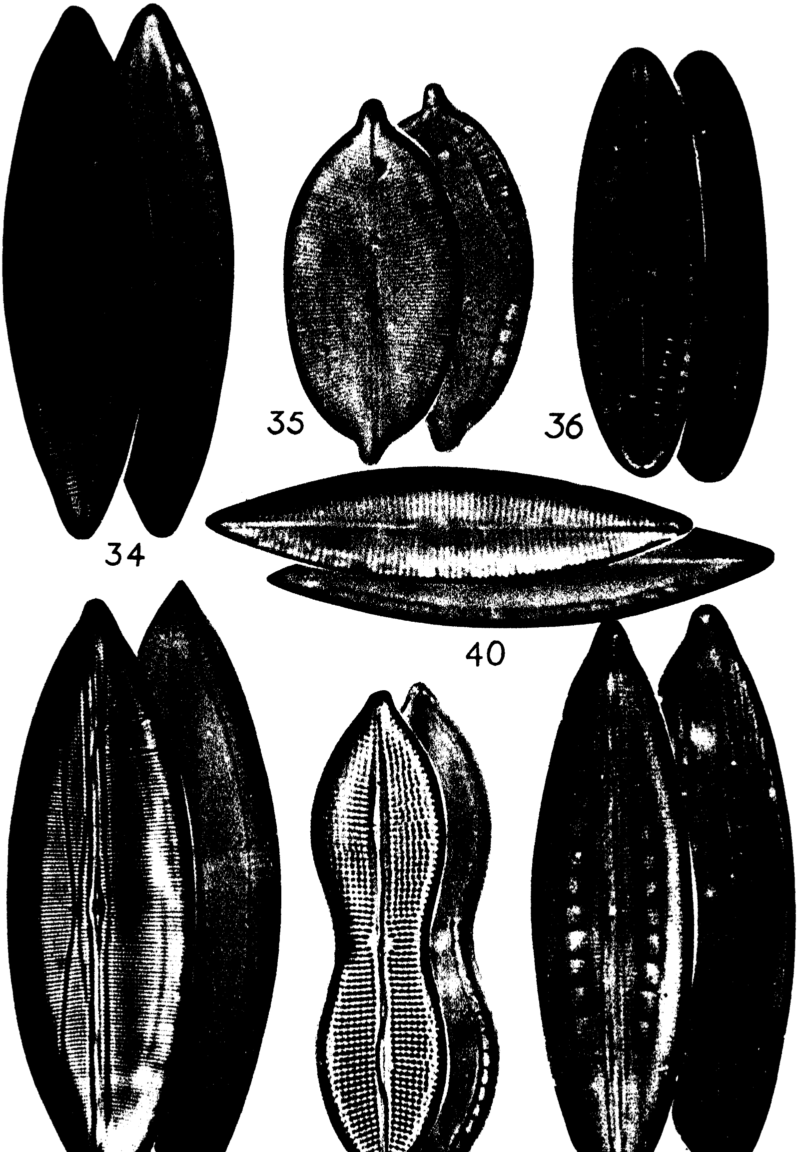
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usque ad marginem percurrens; striis transversalis parallelis, 14–15 in 10μ, marginalibus crasse punctatis, quasi cellulosis, ad centrum valvæ levibus, hic spatium rhomboideum cum margine serrato constituentibus. Ad insulam Chusan in aquis marinis.

Even under a low magnifying power, the deeply cut stauros and the serrated outline of the central zone of the valve, distinguish this form from *M. lancettula*, which it otherwise resembles. The terminal loculi are slightly swollen.

(66) *Mastogloia singaporensis* n.sp. Fig. 25.

Valvis lanceolatis, apicibus obtusis aliquando productis, 50–57μ longis, 13–17μ latis; raphe directa; striis punctatis 28 in 10μ, decussatis; ad aream axillarem angustam cum duis lineis parallelis ad nodulum centralem interruptis; area centralis magna, utrimque dilatata; loculamentis 5–7 in 10μ, 2–3μ latis, non ad apices attingentibus. Ad oras Singapore insulæ, in aquis marinis.

This is a beautiful diatom, resembling *M. decussata*, except for the dimensions of the valve, the large well-defined central area, and the size and arrangement of the loculi, which terminate much further from the apices. In this also it differs from *M. dissimilis* Hust.

The axial area is enclosed between two parallel ribs, which seem to be located on the inner surface of the valve and are interrupted at the central area. It is remarkably constant in dimensions and striation.

(67) *Mastogloia splendidula* Hust. (Hust., p. 468, fig. 886.)

The only habitat given for this species is Miang Besar, Borneo; it also occurs in Nagasaki and the Loochoo islands.

(68) *Mastogloia staurophora* Hust. (Hust., p. 514, fig. 945.)

This species, very rare in the Seychelles, is fairly common in Celebes, where the dimensions differ slightly from those given by Hustedt. Length 25–27μ, breadth 8–8·5μ.

(69) *Mastogloia subaffirmata* Hust. (Hust., p. 527, fig. 960).

Large specimens of the elliptico-lanceolate type, with rostrate apices, were found at Changi, Singapore. Length up to 74μ, breadth 20·5μ, striæ 20 in 10μ, plainly convergent at the apices. The loculi are deeper (3·5μ) and narrower (16 in 10μ) than indicated by Hustedt.

(70) *Mastogloia subaspera v. producta* n.v. (Hust., p. 471, fig. 898.)

Very small specimens occur in the Celebes material; they correspond to Hustedt's diagnosis except that the apices are subrostrate and the dimensions smaller; the alveoli form a characteristic " brick wall " pattern. Length 12·5μ, breadth 7·5μ, striæ 20 in 10μ, slightly radial at apices, loculi 3 in 10μ, 1–1·5μ deep, with concave inner margins.

(71) *Mastogloia sublatericia* Hust. (Hust., p. 479, fig. 900).

The typical broadly elliptical form, with subrostrate apices, occurs in Celebes, together with the following new variety.

(72) *Mastogloia sublatericia v. elongata* n.v.

This differs from the type in its elongated elliptical form, with rounded to subrostrate apices; length 38–63μ, breadth 15·5–22μ. The striæ and loculi are similar to those of the type, except that a larger central area is left free; the loculi are deeply concave and the raphe slightly bent.

(73) *Mastogloia testudinea* n.sp. Fig. 26.

Valvis elliptico-lanceolatis, apicibus obtusis, aliquando leniter productis, 46–82μ longis, 16·5–29μ latis; raphe directa; striis transversis ad polos convergentibus 10–12 in 10μ, longitudinalibus 6–8 in 10μ, obliquis et decussatis, alveolos magnos rectangulares constituentibus. Area axillaris angusta inter lineas rectas alveolorum majorum, centralis dilatata, elliptica. Loculamentis 2–3μ latis 2·5–3, 5 in 10μ, margine concavo, ad polos percurrentibus. In aquis marinis ad insulas Chusan, Nagasaki et Indias Orientales.

Some specimens of this species, with subrostrate apices, might be taken for *M. aspera*, were it not for the fact that the apical striæ are convergent, the alveoli elongated and rectangular and the inner margins of the loculi concave, instead of straight.

(74) *Mastogloia viperina* n.sp. Fig. 29.

Valvis lanceolatis, apicibus acutis, leniter productis, 89–125μ longis, 23–29μ latis; raphe sinuosa ad centrum inflexa; striæ transversales punctatæ, parallelæ, ad polos convergentes, 13–14 in 10μ, punctis elongatis, 8–10 in 10μ, in lineas irregulariter longitudinales dispositis. Apud centrum, nunnulli alveoli elongatissimi. Area axillaris et centralis parvæ; loculamentis ad polos percurrentibus, 4–4·5μ latis, 9–12 in 10μ. Ex holothuriis Indiarum Orientalium.

A large diatom, easily recognised by the extremely elongated alveoli on one side of the central area. A similar formation is found in *M. bahamensis* Cl.; the general structure of *M. viperina* is however finer, although the valves are much larger than those of *M. bahamensis*.

(75) *Mastogloia Yenii* n.sp. Fig. 28.

Valvis ellipticis apicibus capitatis 36–38μ longis, 10–11μ latis; Raphe filiformis, poris centralibus distantibus, unilateraliter inflexis; striis radiantibus, punctatis, ad polos parallelis, 17 in 10μ, puncta 20–22 in 10μ. Area axillaris angusta, centralis magna, ad formam vittæ transversæ usque ad marginem percurrentis dilatata. Loculamentis 4–5 in 10μ, utrimque solum 7, marginibus convexis, 3μ latis. In aquia paludosis Shansi provinciæ.

This species, named after the former governor of the province of Shansi, resembles *M. Smithi v. amphicephala,* in form and arrangement of the loculi, but differs fundamentally both from this species and from *M. elliptica,* in striation and form of the central area. The waters around Tai Yuan Fu, where it occurs, are very hard, so that in habitat, also, it resembles *M. Smithi.*

Summary.

1. The detailed construction of the septate ring of the genus *Mastogloia* has been discussed and an attempt made to classify the fundamental types of loculi.
2. Some difficulties are mentioned in the correct interpretation of drawings made at high magnifications.
3. A number of new species, varieties, and forms of diatoms belonging to the genus *Mastogloia* has been catalogued, together with some remarks concerning hitherto doubtful forms.

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DESCRIPTION OF PLATES.

Plate I.

Fig. 1.—*Mastogloia amoyensis* n.sp. Loculi type 3 × 2000.
Fig. 2.—*Mastogloia balteata* n.sp. Loculi type 1 × 2000.
Fig. 3.—*Mastogloia binotata* f. ovata n.f. Loculi type 1 × 2000.
Fig. 4.—*Mastogloia cebuensis* A. Mann. Loculi type 5 × 625.
Fig. 5.—*Mastogloia cruciata* (L.F.) Cl. Loculi type 5 × 770.
Fig. 6.—*Mastogloia cruciata v. elliptica* n.v. × 1000.
Fig. 7.—*Mastogloia dicephala* n.sp. Loculi type 1 × 2200.

Plate II.

Fig. 8.—*Mastogloia cyclops* n.sp. Loculi type 5 × 1800.
Fig. 9.—*Mastogloia divergens* A.S. Loculi type 5 × 1200.
Fig. 10.—*Mastogloia (?) dubitabilis* Meister × 1730.
Fig. 11.—*Mastogloia elegantula* Hust. × 1000.
Fig. 12.—*Mastogloia fusiformis* A. Mann × 650.
Fig. 13.—*Mastogloia Hustedti* Meister. Loculi type 3 with spines × 1000.

Plate III.

Fig. 14.—*Mastogloia imitatrix* A. Mann. Loculi type 3 × 1000.
Fig. 15.—Details of loculi of another specimen × 1700.
Fig. 16.—*Mastogloia mammosa* n.sp. Loculi type 5 × 1000.
Fig. 17.—*Mastogloia minutissima* n.sp. Loculi type 1 × 2500.
Fig. 18.—*Mastogloia obesa* Cl. × 1650.
Fig. 19.—*Mastogloia orientalis* n.sp. Loculi type 5 × 2000.
Fig. 20.—*Mastogloia pseudocruciata* n.sp. Loculi type 5 × 1000.
Fig. 21.—*Mastogloia serrata* n.sp. Loculi type 5 × 1350.

Plate IV.

Fig. 22.—*Mastogloia robusta v. subconstricta* n.v. × 1025.
Fig. 23.—*Mastogloia robusta v. subconstricta* n.v., another specimen × 805.
Fig. 24.—*Mastogloia rugosa* n.sp. Loculi type 5 × 1000.
Fig. 25.—*Mastogloia singaporensis* n.sp. Loculi type 4 showing shafts × 2000.
Fig. 26.—*Mastogloia testudinea* n.sp. Loculi type 2 × 1000.
Fig. 27.—*Mastogloia mauritiana* (small specimen), Brun. × 1250.
Fig. 28.—*Mastogloia Yenii* n.sp. Loculi type 5 × 1900 and 2000.

Plate V.

Fig. 29.—*Mastogloia viperina* n.sp. Loculi type 4 × 1300.
Fig. 30.—*Mastogloia modesta* n.sp. Loculi type 5 × 1300.
Fig. 31.—*Mastogloia aspera* n.nom. × 1300.
Fig. 32.—*Mastogloia asperula* Grun. × 1300.
Fig. 33.—*Mastogloia lenotiformis* n.sp. Loculi type 5 × 2500.

Plate VI.

Fig. 34.—*Mastogloia celebensis* n.sp. Loculi type 4 × 1800.
Fig. 35.—*Mastogloia mauritiana v. rostrata* n.v. × 1500.
Fig. 36.—*Mastogloia bipartita* n.sp. Loculi type 5 × 1600.
Fig. 37.—*Mastogloia admirabilis* n.sp. Loculi type 4 × 1100.
Fig. 38.—*Mastogloia panduriformis* n.sp. Loculi type 5 × 1500.
Fig. 39.—*Mastogloia lunula* n.sp. Loculi type 3, showing shafts × 2000.
Fig. 40.—*Mastogloia amphipleuroidea* n.sp. Loculi type 5 × 2500.

II.—NUCLEOLAR STAINING METHOD APPLIED TO ANIMAL TISSUES. 535.826.7

By P. N. Bhaduri and C. S. Semmens, King's College, University of London.

Following the introduction of the " Nucleolar Staining Method " (Semmens and Bhaduri, 1939) the technique has been applied to many widely different genera of plants in connection with cytological investigations in this laboratory. Some of the results so obtained have already been published, and others are awaiting publication. These results are very striking and sufficiently testify to the usefulness of the method and its superiority over earlier techniques for purposes of critical cytological observation. Species re-examined by the new procedure have been found to yield far more significant detail than could be observed by other methods (Bhaduri, 1940. etc.). The critical nature of the observation which can be made by the new technique is amply indicated in the above publications and it is considered that no apology need be made here for urging its more extensive use by cytologists.

The importance of the study of the chromosome-nucleolus relationship is being more and more appreciated in recent work, and convincing evidence of the genetical significance of nucleoli is accumulating. Whilst indirect methods (Caspersson and Schultz, 1940) may help considerably in determining the part played by the nucleolus, we feel sure that a wider application of the specific nucleolar staining method will enable such direct observations to be made as will throw light on the genetical behaviour of the nucleolus in general.

In view of the observed superiority of the method, an attempt has now been made to apply it more specifically to animal tissues, where outstanding problems in connection with the hereditary behaviour of the nucleolus are urgently in need of solution. With a slight modification of the usual procedure, preparations were turned out so satisfactorily that prompt publication of the schedule seemed highly desirable. It may be advisable, however, to once more draw attention to the fact that the nucleolar staining method is not a simple counter staining by Light Green but is definitely a specific stain for nucleolin, or at least for the principal chemical component characteristic of the nucleolus. Cytological preparations involving a simple counter staining with aqueous or alcoholic solutions of Light Green, Fast Green, etc., are not to be compared in either nature or quality with those obtained by the present Fuchsin-Light Green technique. Sections of the salivary glands of *Chironomous* or of *Drosophilal*, 25μ to 30μ thick, can

be easily stained in such a manner that the allocation of the green dye to the nucleolus is as clear-cut and specific as the attachment of the fuchsin to the chromosomes.

Since the introduction of the Feulgen reaction it has been customary to hydrolyse animal tissue at 60° C for 4 minutes, though this time and temperature for hydrolysis was only given as a tentative suggestion by Feulgen and Rossenbeck. Though the time and temperature suggested will give satisfactory results as far as a simple Feulgen reaction is concerned, our obser vations have led us to the conclusion that for animal material a temperature of 40°–42°C is high enough to bring about sufficient hydrolysis for the reaction, and at this temperature distortion of chromosomes, as judged by comparison with living material in freshly made smears, is reduced to a minimum. Heitz (1933) claimed that heating to 60°C in normal HCl for subsequent Feulgen staining or cooking the tissue, as practised for making aceto-carmine preparations, faithfully preserves the critical structure of the chromosomes. Our experience, however, has shown us that in delicate materials such as the salivary glands of *Chironomus* or *Drosophilal*, while the gross morphology of the chromosomes may be unaltered, the finer details of structure are definitely affected by heating, or even by the use of certain drastic fixatives such as Carnoy or acetic alcohol. This can always be checked at once by comparing the chromosomes in treated nuclei with those of the living nuclei which can be easily seen in freshly made smears. The use of a lower temperature, 40°–42°C, for hydrolysis, has other advantages besides maintaining faithfully the almost natural appearance of the chromosomes. The liability of sections stripping from the slide is practically eliminated, and the chance of spoiling the preparation by over-hydrolysis, a serious difficulty with the short period of 4 minutes, is considerably reduced by the necessity for a longer period of treatment at the lower temperature.

Although the choice of the fixative to be employed depends mainly on the nature of the tissue under investigation, it has been found that a mixture of 1 p.c. chromic acid and 10 p.c. formalin (Levitsky's fixative) in suitable proportion gives in general very satisfactory results. For instance, whole larvæ of *Chironomus* and *Drosophilal* were fixed in equal proportions of the above two ingredients, and sections 15μ to 30μ were cut and stained. The nuclear fixation in both cases was found to be very satisfactory. The recently introduced chrom-uranium combinations also give equally good results. The two following combinations are recommended for general use :

10 gm. of sodium diuranate are dissolved in 100 c.c. of 10 p.c. chromic acid solution ; keep as stock solution.

1. For fixation use 1 part of stock solution to 9 parts of water.
2. To 7 c.c. of 1·5 p.c. sodium diuranate dissolved in 1 p.c. chromic acid solution, add 1 c.c. of 10 p.c. acetic acid.

SCHEDULES :

A. For sections, e.g. eggs of *Ascaris*, salivary glands of *Drosophilal*, *Chironomus*, etc. :

1. Bring the slides to water and mordant in 1 p.c. chromic acid solution for 1–3 hours. (This mordanting is not necessary for materials fixed in chrom-formalin or chrom-uranium mixtures without acetic acid.)
2. Wash the slides thoroughly in running water ; then hydrolyse in normal HCl at 40°–42°C for 20 to 25 minutes.
3. Rinse the slides in cold normal HCl and then in distilled water. Stain in freshly prepared decolourised fuchsin solution * for 1 to 3 hours (One hour is generally sufficient for staining).
4. Treat the slides with bleaching solution, giving two or three changes, 5 minutes in each.
5. Wash in distilled water and rapidly pass through 50 p.c. and 80 p.c. alcohol to the mordant solution, leave in the mordant for one hour.
6. Wash out the superfluous mordanting solution by three or four changes of 80 p.c. alcohol.
7. Rinse in 90 p.c. alcohol, and then stain for 20 minutes or more in specially made Light Green solution.
8. Wash out the green dye from the cytoplasm by treatment with the differentiating solution, controlling under the microscope. The preparation may be left slightly overstained with green, as this will come out in the final dehydration. Repeat the treatment with the differentiating solution, if necessary, i.e. if the green does not readily clear from the cytoplasm.
9. Dehydrate with 95 p.c., followed by absolute alcohol.
10. Clear in clove oil for 10 minutes, or preferably in dimethyl aniline.
11. If clove oil is used for clearing, wash this out thoroughly by changes of xylol, and then mount in Sira mountant.

B. Permanent preparations of the whole salivary glands of *Chironomus* and *Drosphilal.*

The gland is dissected out and spread quickly on a clean, dry slide. The slide is then transferred at once to a jar of fixative. The whole procedure must be carried out very quickly, as the slightest drying of the gland produces distortion. Fix for ½–1 hour. The slide is afterwards washed thoroughly in running water, and then run through grades of alcohol up to 70 p.c. Leave in the 70 p.c. alcohol for 1–2 hours, so that the tissues may harden. For convenience of working the slides may be left overnight in 70 p.c. alcohol at this stage. When ready for staining bring the slides down to water and then hydrolyse in normal HCl at 40°–42°C for 20–25 minutes. They are next rinsed first in cold normal HCl and then in distilled water. Subsequent procedure is the same as that for paraffin sections described above.

* Particulars for preparing the solutions are given below.

Solutions required :

(1) Decolourised fuchsin solution. (Thomasi-Schiff).

To 100 c.c. of boiling water add 0·5 gm. of basic fuchsin. Allow the solution to cool, then filter, add 10 c.c. of normal HCl and 0·5 gm. of potassium metabisulphite; cork and store in the dark; leave for about 24 hours to decolourise. The solution changes to a faint straw colour. It is best to seal with a cork that has been dipped in paraffin wax.

(2) Bleaching solution :

5 c.c. of normal HCl.

5 c.c. of a 10 p.c. solution of potassium metabisulphite.

90 c.c. distilled water.

(3) Mordant solution :

80 p.c. alcohol saturated with sodium carbonate.

(4) Light Green solution :

Saturate 95 p.c. alcohol with Light Green filter and add 3 drops of pure colourless aniline oil ; shake well.

(5) Differentiating solution :

Use 1 part of mordant solution to 3 parts of 80 p.c. alcohol. Increase or decrease the quantity of mordant solution to suit various materials.

(6) Dimethyl aniline :

An easily obtainable reagent which has distinct advantages over other clearing agents for animal tissues. Details of dimethyl aniline as a cytological reagent will be published by C. S. Semmens in a separate paper.

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III.—TWENTY YEARS OF MICROINCINERATION. CYTOLOGICAL RESULTS.

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The method of microincineration has been in existence for nearly twenty years during which time it has been employed to some extent everywhere, in normal and pathological histology, in cytology, and in zoology. In a contribution which is a tribute to a learned Society that has done so much for the science of structure, it seems justifiable to give a general account of the basic principles upon which this method depends and of the results which it has achieved towards a knowledge of the cell, and to determine what is known to-day of its possibilities and its limitations, of what it can reasonably be expected to yield, and, on the other hand, what it cannot perform.

In such an account, necessarily brief, it will not be possible to describe in detail all the results obtained by this method in normal, pathological, and comparative histology, for papers based upon microincineration already number several hundreds. These pages will have a more restricted aim, namely, to give a critical general survey of its fundamentals and of the results that it has furnished in cytology.

General Significance of the Method of Microincineration.

Biological research has more and more impressed us with the important rôle played in vital processes by mineral substances. In the case of the protein and the lipoprotein complexes of the cell and of the tissues, the mineral elements play an important part in the physiological processes. It has been said that it is the mineral elements which in some way confer life to the protein edifice. It is, therefore, of great interest to determine in what manner the mineral elements are distributed in the cells, tissues, and organs: the calcium, sodium, potassium, magnesium, iron, etc.

Classical chemistry gives little information respecting this distribution; for this purpose, it is insufficiently analytical and topographical. It determines with exactitude the quantity of the various mineral substances contained in an organ, but it furnishes no precise indication of the exact microscopic areas where these mineral elements are located.

In 1918, in the course of a research on the repair of fractures, in order to locate histologically the calcium in the tissues, the Author of these pages made a trial of calcining sections in order to determine precisely the spots where the ash was most abundant. He was thus able to state that, with certain precautions, it is possible to heat to redness an ordinary histological section, fixed upon a slide, and so to destroy completely the organic portions and to reduce it to ashes, without any topographical disturbance of the histological structure. Ash is formed exactly at the spot where the mineral matter occurs in the living tissue. Microincineration yields a faithful image of what may be termed the mineral skeleton of the cells and tissues. It constitutes a general histological method.

Indeed, having determined precisely the technical conditions of the method, the Author discovered, through a careful bibliographical search, that the incineration of organs had already been accomplished. In botany, the French biologist and pharmacist, Raspail,[2] in 1833, over a hundred years ago, pointed out that it is possible to calcine fragments of leaves, vegetable organs, cryptogams, etc., and to reduce them to ashes without disturbing their general structure. Since then, this procedure has been several times employed in vegetable micro-chemistry.

With an object similarly micro-chemical, the German physico-chemist Liesegang [3] showed in 1910 that it is of interest to calcine on a slide sections of animal tissues as a preliminary to micro-chemical analysis. By this procedure, he was able to realize conveniently the operation of "ashing," indispensable in every analysis of organic substances.

On the other hand, neither the botanists nor Liesegang realized in any way that incineration is anything more than a preliminary step in micro-chemical analysis. They did not visualize that it constituted a morphological method of extreme interest, and that it is capable of revealing the precise topographical distribution of mineral substances in the cells and tissues—in a word, to determine the exact arrangement of their mineral skeleton.

Now, it is necessary to-day, to consider microincineration as being much more of a morphological method than a chemical one. Disclosing as it does to the observer the topographical distribution of a special class of chemical element in the tissues, the mineral elements, it is from some points of view a chemical method. But its value chemically is restricted, since, as in the case of iron, it is not capable of distinguishing the various mineral elements. Chemically, the information it affords lacks precision. It gives the total ash, that is, the whole of the oxides, carbonates and phosphates of Ca, Mg, Na, K, Fe, etc., but of these iron alone is distinguishable on account of the red colour of ferric oxide. But as regards the remainder, in the mass of white or grey ash, no distinction is possible between the various chemical elements. Chemically, therefore, the indications furnished by microincineration are very limited. Attempts have often been made to find some characteristic peculiarities that may reveal the true nature of the elements that make up the constitution of the ash. These attempts, up to now, have never given

any result. But should they be abandoned? Probably not, because possibly in treating the tissues before incineration with various reagents, may we not obtain a characteristic ash? But no results of value have so far been obtained in this direction.

Thus, contrary to what Liesegang believed, it is not as a stage in microchemical analysis that microincineration is of interest, but as a morphological method. Demonstrating clearly the position of the mineral elements in the cellular and tissue structures, and giving a definite idea of their relative quantities, it is in reality a morphological method, with all the advantages and none of the inconveniences associated with morphological methods.

Microscopic Constitution of the Ash. The Granules of the Ash: their Mode of Genesis, and their Connexion with the physico-chemical Constitution of the cellular and intercellular Substances.

During incineration, there is no sensible displacement of the mineral substances. With a magnification not exceeding 500 diameters, the image of the mineral skeleton of cell or tissue reproduces exactly the image formed by cell or tissue fixed and stained by the customary histological methods. Under this magnification, the localization of the constituent ashes is very precise and gives an exact idea of the location of the mineral matters in the living tissues.

On the other hand, if one envisages a different degree of magnification, for example, that of a 1000 diameters or more, is localization still exact? For cellular structures of the order of a micron in size, do such localizations of the ash still exist? There are no methods, however precise they may be, that possess these limits. What, then, are the exact limits of localization of the ash? In order to examine this question, it is necessary to envisage the genesis of the ash.

When microincinerations are studied under the highest possible magnifications, it is seen that the ashes are made up of extremely fine granules of mineral matters, the "ash-granules" as they may be termed. What do these granules exactly represent?

If microincinerations of previously fixed tissues are examined, it appears much as though the granules of the ash represent in a single minute agglomeration the union of all the mineral molecules contained in the mass of coagulated protein which has resulted from the fixation. The fixation brings about a coagulation, and the heat causes contraction of the coagula, and induces each one to undergo a kind of shrivelling. The granule of the ash represents the mineral matters enclosed within each elementary coagulum. One conceives, therefore, that the general form of the ashes, and the configuration of the granules of the ash, depend, in some respects, upon the manner of fixation.

If the characters of the granules of the ash are studied in microincinerations of unfixed tissues, that is, placed fresh on the slide, dried on it and afterwards

heated, it may be said that even under these conditions ash-granules are always seen, but they are extremely minute, almost at the limit of microscopical visibility, being hardly two-tenths of a micron in size.

Minute as are the granules of the ash, their dimensions are much greater than those of the protein micellæ. It is, then, not possible to envisage the granules of the ash as being other than the union *en masse* of the mineral matters contained in a minute fragment of protein enclosing a large number of micellæ (or macro-molecules). It may be admitted, with some degree of probability, that they correspond to protein masses of from 1 to 5 cubic microns.

The following succession of events may be regarded as probable. To commence with, the protein substance, dried in the fresh state on the slide, without previous coagulation, forms a continuous layer. At the commencement of heating, this continuous layer breaks up into tiny masses, each of which tends to contract. Under the action of increasing heat, all the protein matters, and particularly those made up of long macro-molecules, in chains, undergo a thermal contraction, varying according to the particular substances and to the principal axes of the molecules. There is a relation between the character of the thermal contraction and the molecular structure.

After contraction, carbonization of the tiny protein mass follows. It seems that contraction continues even after the protein fragment is reduced to a mass of carbon. Finally, all the organic matter is destroyed. The fine granulation of the ash represents the final residue of the destruction by heat of the tiny mass of coagulated protein, together with contraction as a result of the heating. In consequence, for an identical content of mineral matters, the smaller the initial protein mass, the smaller will be the granules of the ash. When in consequence of coagulation by the fixative, the protein masses are more voluminous, the granules of the ash are larger. But the influence of coagulation by the histological fixative is not the only factor. In an organ fixed by a given mixture, there are some areas where the granules of the ash are small, and others where they are large. This depends upon differences in the content of the mineral matters. On this account, micro-incineration is of interest from the biological viewpoint, because it reveals differences in the mineral matters in different areas.

The size of the granules of the ash thus appears to be determined by three factors: (1) the volume of the protein coagula, which depends, among other things, upon the nature of the histological fixative and also on the protein structure; (2) the character and degree of thermal contraction of the protein coagula under the influence of the heat; (3) the mineral matter content of the protein.

If a connection exists between the size and form of the granules of the ash, and the molecular structure envisaged of the protein matter, on the other hand, there does not seem to be any relation between the characters of the ash-granules and the chemical nature of the mineral elements of which they are composed. The form of the ash-granules appears to be determined

particularly by the physico-chemical characters of the protein micellæ and by the technical conditions of their coagulation.

This question of the connection between the morphology of the ash-granules and the mineral constituents of the protein elements is one of extreme importance ; it is, in fact, that of the mode of genesis of the granules of the ash, and is still but little understood. When one reflects upon the extreme complexity and difficulties of the problem, it is not astonishing that much remains to be done on the subject, and our ignorance concerning it is easily explicable.

General Appearance of the Ashes and their Mechanism.

The appearance of the ashes varies to a considerable degree according to the cells and tissues.

These different appearances depend in the first place on the amount of the ash, that is to say, according to the number of granules per unit of surface of the incinerated section. There are some portions of cells or tissues that yield an abundant ash, others in which the ash is scanty. These varieties of " density " of the ash are easily perceived microscopically. It is these that cause the variation in different areas of the spodograms and render them of histological interest. They allow classification of the various cells and tissues according to the amount of ash they contain.

If the appearance of different microincinerations mainly depends on the number of granules in the ash, it does not depend only upon that. It is also connected with the particular size of the various granules of the ash.

When the ash-granules are very small, about two- to five-tenths of a micron, that is to say, at the limit of ordinary microscopical visibility, the aggregate of the ash has the appearance of a regular and homogeneous veil. It is only under a considerable magnification that it can be stated that this apparently homogeneous veil in reality consists of very fine granules.

When the ash-granules are larger (more than half a micron) the appearance is quite different. The light, tangential or oblique, with which we examine, is reflected in all directions by irregularities of the surface of the granules of the ash. Hence, some very diverse effects—chalky, crystalline, etc. With still larger granules, of a micron or more, this effect is exaggerated, and the appearance becomes coarsely crystalline.

In the case of the pneumoconioses, one observes voluminous and coarse granules dispersed in a veil of homogeneous ash composed of very fine granules. The large granules of the ash, of a micron or more, represent exogenous particles of mineral dusts. The homogeneous veil arises from mineral substances enclosed within the normal histological structures of the lung or in the intra-alveolar exudate. It is here possible to make a distinction with comparative ease between ashes which are peculiar and those which are basic or inherent, as it were.

Certain kinds of ash have a translucent horny appearance that is quite peculiar ; these appear homogeneous under the highest magnification, and

are almost bulky. This kind of ash is observed with certain types of bony substance. It may be asked in this case, do ash-granules exist which are so small that they are beyond the limit of microscopical visibility?

The colour of the ash is very variable, but it is usually located at 12 gamma $\pm$ in the white, more or less yellowish, greyish or bluish. Concerning the colour of the ash of microincinerations, the following details may be stated:

Certain kinds of ash are a vivid white, resembling that of chalk, and glitter. Others, also white, have no sparkle and lack lustre. Others, again, are yellowish and somewhat translucent. To what are these variations in the colour of the ash due?

It is somewhat difficult to be precise, but it seems logical to assume that there is a connection between the colour of the various ashes and their chemical nature. It is in the first place a question of absorption of radiations, and secondly a question of chemical constitution. But it is also possible that in certain cases the general colour of the ash depends upon physico-chemical structure and the conditions of diffraction and reflection of light at the level of the ash-granules. All these questions need investigation.

Some varieties of ash are red or yellowish, and are those which contain iron, the iron compounds yielding red ferric oxide. The ashes containing iron have a red colour which is deeper the more iron they contain, and when the iron is scanty the colour is yellowish; these facts are well known in analytical chemistry, and may be transposed without modification to the sphere of microincinerations. The red tint of the ferruginous ashes is specific; no other chemical element gives such a colour to the ash.

Some kinds of ash appear on examination to have a peculiar bluish colouration. This bluish appearance of the ash has been specially studied by Olch (1933) [4] and Olch and Scott (1933) [5] who observed it frequently in cancerous tissues, particularly cancers of the skin. The explanation of this bluish tint, often very marked, seems to be that it is of a purely physical nature.

Ashes made up of large particles diffuse white light in a regular fashion and appear white. When the ash-granules are very small—less than four-tenths of a micron—the diffusion of light at their surface is not regular. The ash-granules, if they form a regular and homogeneous layer, then disperse the light inversely as λ,[4] and show the blue "Tyndall Effect." But for this it is necessary for the particles to have the limit of size mentioned, and that the aperture of the objective should be small. If the ash-granules are of the order of from 0·4 to 0·7 micron, they disperse the red light and diffract the blue; they then show the "blue transmission." C. W. Mason (1931) [6] has given this explanation to account for such blue structures that occur in the course of microscopical examinations.

It cannot be denied that much still remains to be done concerning our knowledge of the mechanism which determines the general appearance and colour of the ash.

Microincineration of Cellular Nuclei. Its Results.

From the initiation of the method, it has been possible to deduce from it a result of interest from the viewpoint of general cytology, namely, that the nucleus almost constantly contains a considerable quantity of fixed mineral matter.[7] Nuceli produce a particularly abundant ash, more so than cytoplasm ; this fact had not been suspected previously. The extreme difficulty, if not impossibility, of isolating nuclei, from the biochemical viewpoint had obscured the fact of the high content of mineral matter in cell-nuclei.

Microincineration has shown that a very high content of fixed mineral matter, especially calcium and magnesium, is one of the characteristics of chromatin. As stated, it is the chromatin particles which contribute the mass of ash in incineration of the nucleus. The morphological picture of nuclear chromatin, as seen in cytological preparations, is reproduced in the spodograms. In the course of mitosis, Scott (1930) [8] has demonstrated the important fact that the chromosomes and the elements of the chromatic figure make themselves evident with a wealth of ash formerly unsuspected. It is possible to follow by appropriate microincinerations the different phases of mitosis. It has also been questioned (Scott, 1932) [9] whether the well-known absorbent capacity of the living nucleus for ultra-violet radiations, and in general for very short radiations (X-rays, according to Lamarque and Turchini),[10] is not dependent upon the content of fixed mineral matter in the chromatin, in particular calcium.

Two Japanese authors (Funaoka and Ogata, 1930) [11] have reported discordant results from investigations on the egg of *Ascaris*. They did not find any marked accumulation of ash in the region of the chromatic areas of the mitotic figure. Barigozzi (1936) [12] who worked with the same material has, on the other hand, completely confirmed the results of previous investigators, Scott in particular. In *Ascaris* in the salivary gland of *Chironomus*, and in a number of amphibian and mammalian organs, the chromatic areas in regions in mitosis yield considerable quantities of ash. This fact may to-day be regarded as being admitted.

One may go further. In the case of the voluminous chromosomes of the sâlivary glands of *Chironomus*, it is possible to detect an alternation of transverse bands. This fact, well studied cytologically by Bauer (1935),[13] is connected with the important question of the genes and their chemical constitution. For in microincinerations, Barigozzi (1937) [14] found a succession of bands very like, up to a point, those which Bauer had studied in ordinary preparations. There are some points in the chromosomes in which the mineral matter is abundant, others in which it is scanty. These facts, still but little known, permit us to hope that microincineration may be utilized in the micro-chemical study of genes and in similar questions of morphological genetics.

Microincineration has likewise shown (Policard, 1934) [15] that the ash of chromatin is almost always perfectly white without a trace of yellow tint. This demonstrates that, contrary to classical opinion, chromatin does not

contain iron, or at least only in such minute traces that the colour of the ash still remains white. For the yellow colour of the ash in the presence of iron is an extremely sensitive reaction; thus, the ash of the mineral matter of a group of three red blood-corpuscles is reddish. This gives some idea of the sensitivity of this reaction.

If chromatin is always rich in mineral matter, the nuclear substance, on the other hand, generally contains very little. Instances occur, however, in which the ash left by the nuclear substance appears to be more abundant. The reason for this is still uncertain. It is clear that microincineration permits of interesting investigations upon the micro-chemistry of the nucleus. The field of research from this viewpoint is still far from being exhausted.

Microincineration of the Cytoplasm and its Differentiations.

In contrast to the nucleus, which constantly furnishes notable quantities of ash, the cytoplasm yields a very variable ash. In certain cases, cytoplasm appears to be almost destitute of mineral matter. By dark-ground illumination it appears to be almost optically empty, or at most it shows some very scanty scattered granulations. In other cases, it yields much ash—more than the nucleus—and if so, it appears as pale grey in the white mass of the cytoplasmic ash, and forms a cavity in the mineral mass of the cytoplasm.

There are also the formations of ergastoplasm of glandular cells, which in particular yield an abundance of ash. The secreting cells of the pancreas are very characteristic in this respect.[16] In microincinerations, a section of a pancreatic acinus appears as if formed of an external mass of ash, exactly corresponding to the regions which in ordinary sections stain with basic stains. The centre of the acinus, that is to say, the excretory lumen and the collective apical areas which contain the refringent serous granulation, only very rarely yields granules of sparse ash; it appears as a black space, almost optically empty. Similar observations can be made on the parotid and other glands.

It has not been possible to make out with certainty if the mitochondria yield ash; in general, it has never been possible to recognize a trace of ash even in the most successful microincinerations. At the level in the cells of the intestine, for instance, where mitochondria are so evident, nothing can be observed that suggests their presence. It is the same for cellular organs in which the mitochondria are more or less modified or are arranged in groups, as, for example, Heidenhain's rods of the renal tubules and the rods of Solger of the excretory ducts of the salivary glands.

Microincineration of Contractile and Vibratile Structures.

Microincineration has been applied in the study of striated muscle fibrils (Tschopp, 1929,[17] Scott, 1932,[18] and others, and more recently Hintzsche, 1938 [19]), and the following results may be regarded as being accepted.

In the contractile fibrils, the mineral matters are located for the most part in the anisotropic segments or dark discs. The clear zones and the

light discs (striæ Z) are very poor in mineral matter. One recognizes in microincinerations images that are a counterpart of those exhibited by preparations stained by the ordinary cytological methods.

In the case of the fibrils of smooth muscle fibres, the question is less clear. In some instances, one can find in them an amount of ash which is greater than that in the surrounding sarcoplasm. In other instances, however, this is not observed; the ash of the fibrils and that of the undifferentiated sarcoplasm are intermingled. Systematic research is required on these points.

The ash left by the fibrils of striated muscle in the form of short rods is always quite white, and without a yellow tint that would indicate traces of iron. It may be asked whether the ash of the anisotropic contractile segments contains in particular potassium. Unfortunately, micro-chemical experiments to test this point have not yet yielded results that are definite and certain; this question remains to be studied. Trials with microchemical reactions on the spodograms under the microscope are technically very difficult and have not given utilizable results.

Between the fibrils, the sarcoplasmic substance always appears poor in ash, except where it is in contact with the nuclei; contrary to the usual rule, the latter appear to contain little mineral matter. On the other hand, the cytoplasm in their immediate vicinity contains much ash. The significance of this arrangement is unknown. With regard to these interesting histo-chemical facts, it is not yet possible to make any deductions on the general physiology of muscular contraction.

Microincineration of vibratile structures has been the subject of several investigations. In the bronchial epithelium, at the level of the thick layer of vibratile cilia which characterizes it, one can detect only the smallest traces of ash. The vibratile cilia if in the motile stage are extremely poor in mineral matter (Policard, 1934).[20] The basal granulations of the cilia appear to yield a somewhat abundant ash. The tract which forms these basal granulations on the surface of the cell is indicated by a line of ash. Scott and Horning (1932)[21] have made analogous observations in the *Opalinidæ*.

It is necessary, however, to be very guarded in the interpretation of these results. It may even be considered that the apical tract of ash is connected with the high content of mineral matter in the cuticle of the cells and in the basal granulations of the cilia, or that there has been a retraction of the vibratile cilia, and that it is the ash-granules resulting from this retraction which give rise to this peculiar appearance.

Microincineration of the Nerve Elements.

Various investigations carried out with the technique of microincineration have yielded information on the distribution of mineral matters in nerve elements (Kruszynski 1934).[22]

Contrary to the usual rule, the nuclei of nerve cells appear to be poor in

ash. They are separated from the surrounding cytoplasm by a distinct thin line of ash which corresponds to the nuclear membrane. In the nucleus, a single portion yields an abundant ash, viz., the well-known chromatic mass. This confirms the opinion that this mass is formed of chromatin, and shows that in reality the nucleus of the nerve cell forms no exception. Its special appearance is linked to its peculiar structure.

The cytoplasm of the nerve elements is very poor in mineral matters; on the other hand, the chromatic substance of Nissl contains an abundance. The Nissl's granules often have a yellowish tint, which leads to the belief that the Nissl substance contains iron. The neuro-fibrils yield no trace of ash in microincinerations. Where neuro-fibrils are plentiful, mineral matter is scanty.

Microincineration of nerve fibres is especially difficult. The myelin melts and causes disturbance. Further, the richness in phosphorus compounds renders incineration troublesome. Nevertheless, in spite of the technical difficulties, it can be recognized that the axis cylinder is not devoid of mineral matters, as one had once affirmed. As in the case of muscular fibres, no connection can be established between the information furnished by microincineration and the mechanism of physiological function.

Conclusions.

The foregoing brief exposition of the subject has settled a certain number of points concerning the application of the method of microincineration to cytology, though much has been omitted. It has not been the intention of the Author to review the whole question; such a review has been done several times, most recently by Hintzsche (1938),[19] in which the reader will find an excellent survey on the histological portion of the question and a very complete bibliographical index to the subject.

From the account given on these points, two general conclusions emerge. The first is that the mechanism of the formation of ash-granules, far from being simple, is composed of a series of factors and conditions dependent not only upon the nature and the quantity of the mineral matters contained in the protein substances, but also upon the constitution and form of the micellæ or macro-molecules of which they are composed. On that account, microincineration raises a whole series of points which are connected with the subject of ultra-structure.

In the second place, it may be stated that, in spite of the obscurity surrounding the mechanism of the formation of the ash-granules, the method has demonstrated a fact of great value from the cytological viewpoint. It has enabled us to establish with certainty a certain number of new facts concerning the nucleus, cytoplasm, the muscular structures, etc.

If microincineration has not yielded everything that had been hoped from the chemical viewpoint, it has been shown, on the other hand, to have undoubted value from the morphological and structural point of view. It is certain that the information it has been called upon to furnish to cytology

and histology can only be augmented in proportion to an increase in the number of the researches. It is to be desired that this method should be more wildly used.

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IV.—THE MICROSCOPE IN BIOLOGY.*

By Adrianus Pijper, M.D., D.Sc., F.R.M.S.

(*Centenary Communication, Royal Microscopical Society*, 1839–1939.)

Popular wisdom has it that history repeats itself. On the other hand, people are wont to say, and it is especially after a period of sudden and great emotional stress that one hears this, that "things can never be the same again." The contradiction embodied in the two popular utterances is of course more superficial than real.

History has certainly repeated itself in so far as this is the second time that this Association holds a meeting in this attractive City. But those of us who were present at that previous meeting, twenty years ago, will readily admit that things are by no means the same again. There have been changes in the City of East London, and, as far as we can judge, they are all for the better. As to our Association, the changes that strike me most are the disappearance, through death and otherwise, of so many members who made that 1919 meeting such a memorable one. I shall not mention names, but I feel the need to state here that I have not forgotten them.

For to me that 1919 meeting here at East London, and of course also the first half of the week which we spent at King Williamstown, has become an outstanding memory. It was the first meeting I ever attended, and it has become one of the most pleasant recollections of my life. I was a complete newcomer to a gathering, largely consisting of people who had made their mark in science, in comparison with which my own contributions seemed too painfully small, and yet I was received and listened to and made to feel one of them in the most natural manner possible. The encouragement thus received on that occasion has lasted for very many years, and the permanent friendly relations which I have since enjoyed with so many of you have been extremely helpful in my further scientific undertakings. I regard this my recent elevation to president's rank as a further proof of your kind intentions towards me, and I wish to assure you that these feelings are entirely reciprocated. May I add that to my mind one of the most important functions of an Association like ours, though it is one that does not meet the eye so readily, is the encouragement and moral support it can give to its younger members?

* **Presidential Address to Section C., South African Association for the Advancement of Science.**

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THE FUNCTION OF A MICROSCOPE.

I have undertaken to address you on the subject of the microscope in biology. Let me at once reassure those who dread that I shall approach my subject through formulas and cryptic expressions such as numerical aperture, Ramsden circle and diffraction image. I promise that I shall make the minimum possible use of such technical terms, and, if you are interested in them, you can always look them up in the "Encyclopædia Britannica." Some slight knowledge of optics, plus an elementary conception of the various light rays and the rules governing their conduct, and a nodding acquaintance with the constituent parts of a microscope will be quite sufficient to follow my argument. I shall also avoid going at all deeply into the history of microscopy, quite contrary to the accepted rules that govern the construction of the traditional presidential address. My own information on the historical side is scanty; I could of course hurriedly have consulted a book on the subject, but I have always been very much impressed by the dictum in *Punch* that when you copy from one book, it is just plagiarism, and only when you copy from twenty books, it is research, and I simply had not the time for twenty books. However, I feel sure there is room for a good deal of original research into the history of the microscope, if only somebody would undertake it without any national bias. At the present moment it is extremely difficult to say to which worker in particular a certain advance in methods is due. Did for instance Leeuwenhoek know the method we now call dark ground illumination? In his results there are definite indications that he did. He may have used it, but he evidently never introduced it, and there is always that mysterious story of the one microscope he refused to demonstrate to his visitors from the Royal Society of London. A problem of a different kind is the story of ultra-violet microscopy. There is little doubt that Koehler, in Germany, built the first apparatus for this purpose, but the practical application and the whole development of the method, including the type of apparatus used, has been so completely the work of Barnard that the vexed question of priority seems to lose all its meaning.

Man has been supposed to be adequately defined as the tool-making animal. And then I would unhesitatingly describe the microscope as man's noblest, supreme, and most far-reaching tool. It would be a fascinating task to unravel the marvellous effects the discovery of the microscope has had on our lives. It would need at least another presidential address to do them justice. Let me, as a pathologist, just bring one aspect to your mind. Would man ever have succeeded in successfully combating so many infectious diseases (and it must be remembered that the majority of human and animal and plant diseases are infectious diseases), if the microscope had not revealed the existence of the small organisms that cause them?

It is a common error of thought that the value of the microscope lies in making things appear bigger. To bring this error home to you I shall have to make a digression. That digression brings me to the discussion of a novel.

In perfectly orthodox circles I believe presidential addresses and novels are regarded as incompatible, they are not supposed to mix well. Perhaps an exception may this time be made, for this exceptional novel. Last year there appeared, written by a Hungarian author, by the name of Harsanyi, a book under the title of "Eppur si muove." The English translation bears the somewhat unfortunate title of "The Stargazer." Those of you who are familiar with Italian will already have guessed that the book is a historical novel dealing with the life of Galileo. Novel or no novel, it is one of the most fascinating books I have ever come across. It is full of delightful touches, some of them so skilfully placed by the author, surely himself a scientist, that one must be a bit of a scientist to appreciate them to the full. Just listen how Galileo puts together his first microscope and starts using it. At first he is terribly excited, he cannot leave the thing alone, and everybody he meets must have a peep through it. Very soon, however, his interest begins to wane, the novelty palls, the instrument is laid on one side and forgotten. The author makes no direct attempt to explain. But have not many of us been through the same process or have we not witnessed it in others? And is not the explanation of the phenomenon at the same time the explanation of the enduring fascination of the microscope to more receptive minds, in more skilful hands? Galileo in the novel, like most beginners, merely looked at the ordinary things he could pick up from his immediate surroundings: a leaf, a small animal, a piece of cloth. He certainly saw things bigger, but his mind was not prepared for the method and his material was not prepared, and nothing came of it. There is more to a microscope than that it makes things appear bigger. One has to prepare the material according to the particular microscope method used, and one must have a mind that is receptive to what the microscope reveals. Ask any demonstrator of a first year class of students who are introduced to microscopes. My own demonstrator used to say that it was as dark in our souls as it was in our microscopes. The man who uses a telescope brings things nearer to his eye, he cannot manipulate his objects, and he can do very little to his telescope. The microscopist handles minute quantities of material, which he has to prepare himself, and their very minuteness complicates his task. He cuts, he teases, he fixes, he stains, he adds chemicals, he has to master many delicate techniques before he can have anything suitable to look at. Then comes the choice of microscopic method to be applied, and there are so many of them that one can hardly be an expert in all. And then there still remains the final difficulty, and that is the correct reception and interpretation of what one sees, taking into account the distorting effect, not only of the previous manipulations of the material, but also of the rays of light impinging upon the object.

All this is merely one of the reasons why microscopists regard the traditional question as to how many times a microscope does magnify as completely beside the point. It is the quality of the picture that matters, not its size, and the quality of the picture depends very largely on the pre-

paration of the material and the application of the most suitable kind of illumination. No microscope working with ordinary light will ever be able to employ a higher useful magnification than about 800 times. To go beyond that merely results in blurring the picture. This automatic limitation of magnification has not proved to be such a drawback as it might appear. When microscopists found they could not go higher, they exerted themselves to do better with the means at their disposal, and they started inventing supplementary methods. The result has been that the microscopist of to-day has so many methods at his disposal that one can really speak of an embarras de richesses. The unfortunate part is that one also needs considerable riches to avail oneself of all of them.

This considerable expense connected with the more involved forms of microscopy is probably one of the reasons why, for instance, ultra-violet microscopy has not got beyond the laboratories of the British Medical Research Council. I am not losing sight of the fact that the whole apparatus would probably not have existed if it had not been for the genius and perseverance of Barnard, coupled with the technical devotion of Beck. But the original outlay for the higher and more specialized forms of microscopy, of which I shall give you some examples just now, is rather formidable and not within the reach of private individuals or even the smaller official institutions.

Ultra-violet Microscopy.

Microscopy by means of ultra-violet light is a perfectly natural development of microscopy with ordinary light. The real power of a microscope is not expressed in terms of magnification, but in terms of resolution. The resolution depends on the objective alone, for the eyepiece merely serves to magnify the picture to a convenient size, but does not add anything to the resolution. Now by resolution we mean how close together two points in the object under observation can be to be still observed as two points. The distance between two such points is a measure of the power of resolution of the given objective. Now in the simplest possible words this power depends on two factors only. One is what is called the numerical aperture of the objective. It means more or less how close you can bring your front lens of your objective to the object you are looking at and still get a picture. The closer you get the greater the resolution, but glass being what it is, you cannot get closer than a certain distance, and that puts a natural limitation to the factor called numerical aperture. The other factor is the wavelength of light, and the shorter wavelengths give the greater resolution. In simple terms, the amount of detail that one can see, stands in inverse proportion to the wavelength of the light one uses. Visible light stops somewhere at wavelength $0{\cdot}5\mu$. If one had visible light of a wavelength $0{\cdot}25\mu$, one would see twice as much detail. Light of that short wavelength is called ultra-violet light, it is invisible, but it does affect photographic plates. It is not

refracted by glass, but it is by quartz. And so, by replacing visible light by ultra-violet light, and glass lenses by quartz lenses, and the eye by a photographic plate, Barnard has succeeded in photographing bodies at least twice smaller than what the human eye can see. It has probably taken him more years to do it than it has taken me minutes to explain it. The difficulties of getting suitable light, and suitable quartz lenses, and then again, and this I want to emphasize, a suitable preparation of the material, were enormous, but the results were well worth it. Barnard has produced photographs of virus bodies that nobody had thought could be made visible to the human eye. Their existence was suspected, but there was no proof whatever, and most people who believed in the so-called particulate nature of viruses were careful to add that these particles which were the virus were too small ever to be seen. Barnard, by increasing the scope or resolution of his microscope by 100 p.c., succeeded in making them visible on the photographic plate, and he is now considering, by using ultra-violet light of still shorter wavelengths, to still further increase the power of his microscope.

Infra-red Microscopy.

The obvious counterpart of ultra-violet microscopy, which achieved greater resolution by employing shorter wavelengths of light, is of course infra-red microscopy. The aim of infra-red microscopy is not greater resolution, but greater penetration. To many of you the words infra-red rays will recall memories of photographs of the coast of France, photographed from the coast of England on a foggy day, the particular virtue of infra-red rays being that their long wavelength permits them to find their way, unhampered, round fog particles. It is probably less well known that this ability to ignore small particles has led to the use of infra-red rays in microscopy. For ordinary microscopic work one, as a rule, prefers to place the thinnest possible slices of material on the stage of the microscope. Thick slices absorb and hold back too much of visible light, and the impression gained by the eye will just be one of general turbidity. The position, however, becomes quite different if the visible light is replaced by infra-red rays. Now the infra-red rays will not be affected by the numerous small particles causing the general turbidity, but only by the more solid and well-defined and more compact structures present in the preparation. Here, again, infra-red rays being invisible, one has to rely on the photographic plate for the picture. The procedure is somewhat as follows: one focuses the preparation by means of ordinary light as best one can, places the infra-red filter between microscope lamp and microscope, puts the special infra-red sensitive photographic plate in position, and experiments with exposures until the photographic plate on development shows definite structures. I found it, for instance, possible to photograph bacteria in the depths of agar media, in which they were quite invisible by ordinary microscopic methods on account of the turbidity of the agar medium. The method has already been extensively used for histological

purposes and very curious pictures of thick slices of tissues have been obtained. The microphotographs are sometimes difficult of interpretation, but there is no doubt that here is another field of research in microscopy which leads at once into completely uncharted fields.

Dark Ground Microscopy.

There is one special microscopic method that has always had rather a hard time, and is still struggling for recognition. I refer to what is known as dark-ground microscopy. It is true that Barnard's ultra-violet microscopy in essence also is a dark-ground method, but with ordinary light the dark-ground principle never has quite got into its own. From personal experience I am convinced that a proper application of the dark-ground method with visible light is capable of solving problems that baffle ordinary microscopic methods. In dark ground one has the inestimable advantage of using live material in normal surroundings, avoiding the injurious processes of fixing and staining. For sheer beauty the pictures are unsurpassed, silver white shining micro-organisms can be seen moving against a velvety black background. Especially when the microscope is provided with binocular tubes, and a stereoscopic picture can be produced, the spectacle of a deep dark layer of fluid, in which bright and glittering organisms and cells and particles move restlessly about, is to my mind greatly preferable to even the Victoria Falls on a moonlit night.

Dark ground microscopy is based on a phenomenon with which we are all familiar. A ray of sunshine penetrating through a narrow slit into a darkened room, when looked at sideways, shows up millions of dancing particles of dust, which are much too small to be seen otherwise. Dark ground microscopy means the application of this phenomenon to the microscope. And here we at once encounter the difficulties attached to the method, and the reason why it is not in more general use. The light has to be brought to the preparation on the miscroscope slide in such a way that it passes through the material without being able to enter the objective, in other words, so that the observer looks at it sideways. All the light that the observer may see must be as it were reflected by the particles in the microscope field. For this purpose special substage condensers have been designed which throw the light very obliquely through the preparation. In order to get as much light as possible, the condensers are so designed that oblique rays enter the preparation from all sides, in circular fashion, and they are brought to a very narrow focus. This narrow focus concentrates all the light, and it is only in this focus that the phenomenon of dark ground illumination shows itself. It follows that accurate centration of light source, condenser, and microscope lenses becomes of supreme importance, apart from the correct adjustment for height of the condenser with its small focus. Here lies the source of the many failures which have made the method unpopular. It requires endless patience and an experienced hand to get the best out of a dark ground micro-

scope, and there hardly is a second best. Slipshod methods mean complete failure. A well-managed dark ground picture, apart from the advantages already mentioned, increases the visibility of details because they show up white against a black background.

The Construction of Microscopes and Lamps.

I have said that the dark ground method has not found great favour with microscopists on account of its intrinsic difficulties. But here not all the blame should fall on the shoulders of the microscopists. The makers of microscopes are also partly guilty. There are still far too many microscopes put on the market which do not make adequate provision for easy and accurate centration of condensers and objectives. A revolver for carrying objectives is a very half-hearted attempt at centration, and in most microscopes the substage arrangements are treated like proverbial stepchildren. It is probable that mass-production has something to do with this, but microscope builders do seem to belong to the conservative party. Microscopes in common with motor cars are becoming more and more streamlined, but essential improvements lag behind. Let me give some illustrations. A device for accurate centration of objectives and condensers should not be regarded as a luxury. There is nothing more vexing in microscopic work than to "lose your place," and is there any firm that has invented a really satisfactory method to find it again? There are a few objectives on the market now with adjustable iris diaphragms built into them so that one can vary the numerical aperture at will. Is it asking for too much if we want more of them? Talking of convenience, I do not want to go so far as the old professor who had a support for his cigar attached to his microscope stand so that he could look down and smoke in comfort, but, seeing that motor cars have sliding gear changers nowadays, is it impossible to build eyepieces that allow a gradual increase in magnification? Binoculars are still regarded as an "extra," and it is only quite recently that makers of microscopes have discovered that it is more comfortable to look through a microscope at an angle than straight down. Has any designer of microscopes ever enquired from his customers what alterations they would like? The general pattern of microscopes has changed very little from the early days onward, if one looks at pictures in history books. The old and rather futile controversy whether a microscope should have a horseshoe base or find stability on a tripod seems to have been settled. Lenses used to be built empirically, they are carefully calculated beforehand now. Would it not be possible to apply the same sound engineering principle to the stand? Why should a microscope stand consist of one upright pillar, to which a series of brackets are attached which carry the various parts? Surely a microscope stand needs stability and can that be expected where all its delicate parts are supported at one point only, instead of at three? At the present time the slightest pressure on the stage throws the whole picture out of focus. I am not referring

now to the enormously heavy and solid and bulky apparatus that the larger firms have recently put on the market, and which are far too expensive and perhaps not very useful to the ordinary worker. It is the ordinary microscope stand that we all buy to which I would like to see applied some elementary principles of engineering.

The criticisms expressed must not be taken to mean that I do not appreciate all the good things the designers of microscopes have given us. It is, I suppose, just human nature to accept what is good without comment, and to dwell on things one would like to see altered. One of the good points about modern microscopes is their internationalness. What I mean is that nowadays a high degree of international standardization has been reached. Practically all modern objectives are calculated for a tube length of 160 millimetres, tubes are generally of such uniform width that they will take any eyepiece, and finally, the screwthreads of practically all makes of objectives are similar and will fit any standard microscope. Here most construction engineers have a lot to learn from the firms that build microscopes.

There are still one or two things I would like to say about microscope lamps. Nobody nowadays uses daylight for microscopic purposes. The advantages of artificial light, especially electric appliances, are great. The surprising part is that so little thought is given to the construction of these lamps. Far too many of them allow a good deal of the light to stray into the observer's eye. This narrows his pupils, and makes his retina less sensitive to the fainter light that his eye receives from the microscope. It is not a minor point, and it also illustrates the little thought that is so often bestowed on such matters. A very serious shortcoming, I think, of electricians is that they have not succeeded in producing a lamp that is really suitable for dark ground work. Sunlight is still several times stronger than the best electric lamp that can be used for such purposes. Such a lamp should be easy to handle, and free from danger. It should have a very small area of very high intrinsic brilliancy. Dark ground methods depend on the contrast between brightly lit particles against a black background. The brighter the light, the more detail will become visible, and I have seen things show up clearly when I used sunlight for dark ground microscopy that remain hidden with any other kind of illumination. Sunlight has the disadvantage of not being always available, and it requires a heliostat for observations of any duration. But no lamp so far can compete with it as regards brilliancy and efficiency, and as long as this is the case, and no simple and efficient artificial light source of similar power is put on the market, so long will the further progress of dark ground microscopy be held up. This is a great pity, for dark ground examination, especially for the examination of objects in their natural liquid surroundings, has advantages that are unsurpassed.

Fluorescence Microscopy.

This problem of suitable microscope lamps brings me to another chapter of advanced microscopy, which is called luminescence or also fluorescence

microscopy. Although the first patents were taken out several years ago, it is only quite recently that the method has been taken into practical use. Here too the lack of an adequate light source has held up progress. Fluorescence, as everybody knows, means that many substances have the capacity, when irradiated with light of one wavelength, to send out light of a longer wavelength. In practice the most effective way is to use invisible ultra-violet light for the irradiation. The fluorescence of the substances under examination then sends out visible light, and the colour of this visible light is often characteristic of the substance irradiated. In order to apply the method to the microscope, a very powerful source of ultra-violet light is needed, and this need has not been quite satisfactorily filled so far, except perhaps in the case of very high priced apparatus. Also, the ultra-violet light can only be brought to the object on the microscope stage by means of a quartz condenser, and naturally one has to use quartz microscope slides. The rest of the microscope equipment, which has merely to convey visible light to the eye, can be left as it is.

At first it seems as if the use of such a fluorescence microscope would be limited to the examination of materials that are capable of fluorescence as such, the phenomenon known as auto-fluorescence or primary fluorescence. In this field important observations have been made, and with the increasing number of substances that are found to exhibit characteristic fluorescence, the method is becoming increasingly useful. It has already led to the discovery of new kinds of cells in the animal organism, which could be picked out by their curious fluorescence. Similarly, some vitamins possess a characteristic fluorescence and their origin and development can be traced by means of fluorescence microscopy. It has been found that live carcinomatous cells exhibit a fluorescence which is not met with in other cells. For examinations of large pieces of material it is sometimes more advantageous not to admit the light through a substage condenser, but to send it down from above, following the method which is called vertical illumination, for which particular kinds of condensers called epi-condensers have been constructed by some firms. There is a good deal of spade work yet to be done in this field, the normal fluorescence of normal cells and tissues of both animal and vegetable origin still have to be worked out, and probably this new method will be found to give its maximum return only when a spectrometer is added to the microscope in order to analyse the exact constitution of the fluorescence colours. However rich the harvest here is going to be, and it must be remembered that especially plant cells are capable of producing a large variety of fluorescence colours, still more is perhaps to be expected from another application of the principle of the method, which is called secondary fluorescence. Observing primary fluorescence has the advantage of dealing with untreated and undamaged cells, for secondary fluorescence the material has to be prepared and treated beforehand, not however with fatal results to the cells in all cases. The preparation of the material consists of treating them with weak solutions of certain dyestuffs. These dyestuffs

are capable of fluorescence to a very marked degree. They have, moreover, very often a selective action, i.e. they have preferences for certain cells, or for certain structures in cells. These dyestuffs are called fluorochromes and there is already a large variety of them. By their judicious application it is possible to induce all kinds of cells or particles or structures, which otherwise would have remained hidden to give proof of their presence by making them show up in bright colours whilst the background remains dark. It is, for instance, very much easier to spot an isolated tubercle bacillus or leprosy bacillus in a mass of debris by means of fluorescence microscopy than by any other method. There are several virus bodies for which selective fluorochromes have been found, which make them show up in bright colours in the cells containing them. The method has already been used to show that chinin and atebrin, our remedies against malaria, act by directly penetrating the bodies of the parasites and not in any indirect manner. Here good use has been made of the fortunate fact that both these substances, chinin and atebrin, apart from being good remedies, are also fluorochromes. Recent observations go to show that cancer cells contain small bodies which only become visible by the application of a suitable fluorochrome. But it is not only in pathology, with which I am of course more conversant, that secondary fluorescence has proved its worth in microscopy. In physiology, certain fluorochromes have been used to study the penetration, transport, and retention of dissolved substances by plant cells, and there is no doubt that further applications will be found. In these matters we are only at the beginning, and there are still foundations to be laid.

The Electron Microscope.

I have specifically been warned that I must not keep you here longer than fifty minutes. This address therefore cannot be a complete review of modern microscopic methods. But my account would be regarded as very incomplete if I did not make mention of the instrument that first became known to many of us some time ago through the columns of the daily press. It was made to appear as if through the advent of this "super-microscope" also called "electron microscope," we would all have to scrap our ordinary microscopes and take to the new instrument, for which magnifications of a hundred thousand times seemed to be a perfectly easy matter. Such a magnification would enlarge one red blood cell to a disc of a diameter of seven metres, not a very convenient size to handle. Anybody, however, who has studied the available scientific literature on the subject will admit that his original feeling of incredulous surprise has made way for a feeling of profound admiration for the workers who have succeeded in making this miracle possible. Electrons, although in this apparatus they are used for microscopy, have of course nothing to do with light. They are extremely small particles, smaller than the hydrogen atom, they carry a negative electric charge, they are produced by electric discharges in evacuated tubes, and they

can be made to travel at an enormous speed. They travel in a straight line, as long as they do not bump up against anything, and therefore the electron microscope has to work in a high vacuum. The electrons being electrically charged, a suitably arranged electro-magnet will deflect them from their course. The secret of the electron microscope is that electro-magnets of particular shape take the place of the lenses in the ordinary microscope. A beam of electrons can be brought to a point or focus through the action of suitably placed electro-magnets. An electron microscope has a substage condenser, an objective, and an eyepiece, all worked electro-magnetically, and focusing is done by altering the strength of the electric currents going through the electro-magnets. The advantages are obvious. As there are no glass lenses, the question of numerical aperture disappears. For the same reason diffraction rings cease to interfere with the images obtained. Because there is no wavelength to be considered, the limitation inherent in that factor completely falls away. There is definitely no limitation to the magnification of an electron microscope, figures of a million times have been seriously mentioned, and magnifications of thirty thousand times have actually been reached. A drawback is that electrons not being light rays, the resulting picture cannot be seen directly, but must be made visible by catching the electrons on a fluorescent screen, where a picture will be formed, or on a photographic plate which then can be developed in the ordinary way. The pictures of bacteria that have been published show details inside their bodies that are quite beyond the powers of the ordinary microscope. Quite recently photographs have appeared of bacterial flagella which show very convincingly that the electron microscope can record these delicate structures in an unstained condition. In this case magnifications of several thousand times were easily reached and still give excellent photographs.

Although I therefore have great faith in the future of this new method, also in biology (for metallurgical purposes it has been in use for quite a time), I cannot withhold from you the drawbacks of the supermicroscope. Electrons have a deleterious effect on living cells, the preparations have to be exceedingly thin, one cannot use glass slides but has to use dried preparations on a thin collodium film, and with the usual type of supermicroscope these preparations have to be photographed in a vacuum. Recently a new method has been described which at least circumvents this last objection. Instead of imitating an ordinary microscope, an extremely narrow beam or fine pencil of electrons, which is produced again by a suitable arrangement of electro-magnets in a vacuum, is sent through the preparation to be photographed. This fine pencil of electrons leaves the apparatus through a thin collodium window, and travels a very short distance through the air before it reaches the preparation, which therefore can be kept in the ordinary air. The pencil is made to travel along parallel lines through the preparation, and underneath is a photographic plate which travels in harmony with the pencil, but at much greater speed. The pencil thus etches a very much enlarged picture of the structure of the preparation on the photographic plate.

Of course the method owes its possibility to the extreme fineness of the pencil of electrons. The super or electron microscope may not be an instrument for daily use yet, but there is no doubt that it holds enormous promises for the future.

Microscopy and Research.

What is known as the field of a microscope is a very small area, at best it extends over a few square millimetres. I have dwelt on the various aspects of this narrow domain for quite a time, and for fear of being branded as a man of a very narrow outlook I shall now ask you to follow me into a wider field. In truth, my chief aim to-day is not so much to enlighten you about various microscopic methods in detail, as to impress upon you that microscopy is a science in its own rights, worthy of being pursued for its own sake. I want to make you look upon a microscope, not just as a tool that comes ready made from the dealer in optical instruments, but as a piece of apparatus that can come alive in the hands of its user, an instrument that is capable of nearly unlimited development, and which can be the object of research by itself. May I say that this aspect of microscopy is rather neglected in South Africa? And that I always have been, and am, and probably shall be the only South African Fellow of the Royal Microscopical Society? On two occasions I have made tentative suggestions to professors of biology that I should be permitted to lecture to their students on the use and perhaps, more suitably, the abuses of microscopes, with perfectly negative results. I have a feeling that this indifference towards microscopy as a self-contained science, with research objects of its own, is merely a symptom of that somewhat too practical attitude that pervades most South African scientific work. I know that with this remark I have got on to very thin ice, or, as we rather should say in this country, on to caving ground. Perhaps I had better follow the lead of that lady motorist acquaintance of mine, who made a point of always taking all street corners at the highest possible speed, because she had heard that the greatest danger of accidents was at such corners.

It is undoubtedly true that in South Africa we are surrounded by a number of practical problems that cry out for solution. It is only natural that research workers are powerfully attracted by these problems. There is in addition the atmosphere of competition and rivalry which is a feature of modern times, and which again spurs on the scientific worker towards quick returns and speedy solutions. Research in agriculture and industry has to be practical in order to keep in step with the large strides that are being made everywhere. Hardly have we set up an institute for wool research or the very existence of wool farmers is threatened by the production of artificial wool. We have barely found methods to combat horse-sickness or the horse is being superseded by mechanical traction. Whilst our grass researchers have not quite settled yet what is the best kind of grass to go in for under various conditions, the soil erosion department lifts up a warning voice and

tells us that unless something is done there will be no soil left to plant the grass on. Our natural resources seem to be dwindling or lose their value whilst we are still engaged on studying them and finding ways and means to preserve them. It is then not to be wondered that most of our scientific work is animated by a spirit of haste, by a burning desire to do something practical. Our attention goes out towards practical application, at the end of the work there must be "something to show for it." The professor can no longer just profess his science, he is called upon, every day of his life, to give practical advice, to help in solving practical problems. The microscope is just a practical tool, like the carpenter's hammer.

It is far from me to condemn this attitude. I have the profoundest admiration for my fellow scientific workers who apply their gifts and energies to these grand efforts to benefit the country and its inhabitants, in so many instances with such conspicuous and far-reaching success. But I would like to use this opportunity to put in a word for an aspect of scientific effort that I am afraid is in danger of being crowded out. I cannot help deploring that what is usually called pure, or fundamental, or academic research is not getting the recognition which, to my perhaps somewhat biased mind, it deserves. It may be due to a defect in my intellectual make-up, but it has taken me some time to get used to the idea that the august body called the Research Grant Board comes under the Department of Commerce and Industry. I personally have the best of reasons not to say anything in criticism of this body, but are we not agreed that if I apply for a grant from that source, my chances of success will be greatly enhanced if my application can be worded so that it appears as if I was aiming at growing bigger and better oranges, or at doing away with some agricultural pest? And would not my chances of a favourable reply be diminished if I stated frankly that I just wanted to do a piece of pure research, just for the sake of research, the famous piece of research that will never be any use to anybody? There is room and opportunity and need for the kind of research that does not directly lead anywhere, especially in biology. And this kind of research should get the same amount of support and encouragement that is now so largely accorded to research in its more practical application. It is the kind of research that cannot be directed, but it should be fostered. I regard it as a sign of the highest culture, same as art, and it needs leisure and security. The mentality that discovers or invents something new is not always the mentality that can think out practical applications. The practical side often simply does not interest such people. I believe it is on record that the man who invented the electro-magnet, the conception which in the mind of somebody else gave rise to the practical application called an electro-motor, refused to go and see that electro-motor work, he simply was not interested. It would be easy to multiply examples of this kind. It might be objected that my contention that pure research should be publicly supported is all very well and good where geniuses are concerned. In order to counter this objection, I may perhaps be permitted to quote an example, *si parva licet componere magnis*,

from very much nearer home. At the previous meeting of this Association in this town, in 1919, I had the pleasure of demonstrating a new method I had just invented of measuring the diameter of red blood cells. It found favour with the Association, and I am pleased and grateful to relate that the Research Grant Board, mainly, I believe, on the recommendation of this Association, gave me liberal financial support for the further development of the method. But however good the new method was, for years it was practically completely ignored, simply because it did not seem to matter from a practical point of view whether a person's blood cells were a shade bigger or smaller than normal; until somebody discovered that the diameter of red blood cells is an important factor in the diagnosis of certain diseases of the blood. And then suddenly the despised method became extremely popular and several firms started building apparatus for the practical application of the method, which is now in daily use in most medical laboratories. The point I wish to make is that here we have an example of a new method being worked out merely for the fascination of the thing in itself, which, however, years afterwards meets with genuine appreciation by practical people. And also that a certain amount of practical, i.e. financial support, is often essential for the proper development of an idea. It is regrettable that modern research, whether it is practical or academic, has become so dependent on expensive apparatus. You may remember that when Mrs. Einstein was shown over a new observatory and asked what the truly gigantic mirror of the telescope was for, she was told that it was for studying the universe. "Oh," she said, "my husband does that on the back of an old envelope." To most of us the backs of old envelopes would not suffice as facilities for research. A good deal more is needed, and as scientists are notoriously poor, they have a claim to public money.

Now it cannot be denied that a good deal of public money is being spent on research. It is merely against the stress that is so often laid on the need for immediate practical returns that I would like to utter a mild protest. I have just made an effort to present the science of microscopy to you as a science by itself, capable of higher development, and offering wide fields for research. At the same time it is rather a detached science, from which no immediate practical results can be expected. The pure science of microscopy looks at things just to see how they look under various conditions, but not to find out what they could be used for. It tries to make things visible that are hidden from our eyes, but it is not very interested in their purpose. It rejoices more in the invention of a new method, than in the discovery of new facts, although it is pleasantly aware of it that a new method invariably leads to the discovery of new facts. It does not mind being useful to the practical worker, but it does not set out to be. It is essentially what is called long term research. It is this aspect of microscopy that I have tried to bring to your attention. Many of you use microscopes, but few of you are microscopists. The fact that so many of you use microscopes should make you pause and consider whether you really get the best possible out of microscopy.

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And whether some more time and energy might not be devoted to microscopy as such, and some facilities should not be given to those who possess an aptitude for scientific work of this kind? Perhaps a beginning towards a better appreciation could be made by instituting microscopy as a subject to be specifically taught to the young biologist. The present system under which they just pick it up as they go on is rather haphazard and cannot lead to a proper understanding of its possibilities.

Ladies and gentlemen, in looking back on this presidential address, I feel that you had probably expected me to say less about miscroscopical methods and their scientific status and more about actual observations through the microscope. May I remind you that an address on microscopy must necessarily be written during the times when the microscopist sits back, and relaxes, and does not look through, but at, and perhaps even a little beyond, his microscope.

ABSTRACTS AND REVIEWS

BOTANY.

(*Under the Direction of* J. Ramsbottom, *O.B.E.*, D.Sc.)

Cytology.

Cytology of Antirrhinum.—H. Ernst ("Zytogenetische Untersuchungen and haploiden Pflanzen von *Antirrhinum majus* L.," *Zeitsch. f. Bot.*, 1940, **35**, 161–89, 1 pl., 3 text-figs.). Meiosis was studied in a haploid strain of *Antirrhinum majus*. In the prophase there occurs a stage analogous to the pachytene of normal diploid meiosis, the eight chromosomes of the haploid complement pairing up with each other. In the subsequent diakinesis univalents, bivalents, and multivalents are formed, and the modes of their subsequent distribution to the two daughter nuclei are described. The behaviour of the chromosomes in seedlings derived from a cross between haploid and diploid plants was studied; in 34·57 p.c. of the cases delayed alterations of the chromosomic constitution were observed, this being due to the peculiar conditions of pairing during meiosis. I. M. L.

Development of the Embryo-sac in Convallaria majalis.—H. Stenar ("Über die Entwicklung des Embryosackes bei *Convallaria majalis* L.," *Bot. Not.*, 1941, 123–8, 5 text-figs.). In *Convallaria majalis* the original nucleus of the embryo mother-cell undergoes a reduction division to form two daughter nuclei, which are then separated by a cell wall. Then follows a homotypic division giving rise to four cells, the membranes between the upper and lower pairs being later resorbed to give two binucleate cells. Of these, the lower (chalazal) cell becomes four-nucleate by simple division, while the two nuclei of the upper (micropylar) cell eventually disintegrate. Further development takes place in the remaining chalazal cell, which becomes eight-nucleate and ready for fertilization in the usual manner. This type of development is similar to that found in *Allium* and *Scilla*.
I. M. L.

Morphology and Reproduction.

Embryo Sac.—F. T. Wolf ("Macrosporogenesis and the development of the embryo sac in *Yucca aloifolia*," *Bull. Torrey Bot. Cl.*, 1940, **67**, 755–61, 15 figs.). In *Yucca aloifolia* L. a tetrad of macrospores is produced having a "T" arrangement, the micropylar pair lying side by side. The chalazal macrospore forms the embryo sac which develops normally and at maturity is 7-celled. The chalazal portion is tubular and possibly serves as a haustorium. F. L. S.

Torreya Embryogeny.—J. T. Buchholz ("The Embryogeny of *Torreya*, with a Note on *Austrotaxus*," *Bull. Torrey Bot. Cl.*, 1940, **67**, 731–54, 44 figs.). In

Torreya nucifera and *T. californica* there are generally three archegonia, in *T. taxifolia* usually only one. Other points made during this investigation of the embryogeny of these species are: that none forms a ventral canal cell; the megaspore membrane is absent or so thin that it is usually not observed; in *T. taxifolia* the pollen tube is in intimate contact with the archegonium throughout its development, invading and digesting the neck cells and upper part of archegonium; in *T. nucifera* the neck cells persist, the cytoplasm of the male cells is usually found in the upper portion of the egg and does not contribute to the cytoplasm in the zygote.

F. L. S.

Anatomy and Morphology.

Formation of Septa in Fibre-Tracheids.—P. A. Vestal and M. R. Vestal ("The Formation of Septa in the Fibre-tracheids of *Hypericum Androsaemum* L.," *Bot. Mus. Leaflets* (Harvard Univ.), 1940, **8**, 169–88, 2 pls.). The development of the fibre-tracheids is normal in that they undergo elongation and maturation of the secondary wall. However, the protoplast remains active and usually undergoes division at a right angle to the original division of the cambial initial. The septum is formed in the region of the cell plate and the daughter nuclei migrate to the central region of the newly formed compartments. The formation of septa is apparently not confined to any particular region, but may occur in any part of the secondary wood formed during a single growing season.

B. J. R.

The Chemical Nature of the Intercellular Substance in Wood.—H. E. Dadswell and D. J. Ellis ("Contributions to the Study of the Cell Wall. 3. The Fibre-Bonding Materials and their Importance in Pulping," *J. Coun. Sci. Ind. Res.* (Australia), 1940, **13**, 290–8, 2 pls.). Thin sections of wood can be delignified without causing their maceration, the resulting sections being the equivalent of holocellulose. Maceration of such sections can be accomplished by means of dilute sodium hydroxide. This shows that there are two main constituents of the middle lamella zone which act as cementing materials bonding the individual fibres together. One of these is lignin. The chemical nature of the other is under investigation.

B. J. R.

The Chemical Nature of the Intercellular Substance in Wood.—A. W. Mackney ("An Attempt to Isolate the Fibre-Bonding Material from the Holocellulose of *Eucalyptus regnans* F. v. M.," *J. Coun. Sci. Ind. Res.* (Australia), 1940, **13**, 299–304). It is suggested that the non-lignin, fibre-bonding material is a non-furfural yielding substance present to the extent of 2·8 p.c. of the holocellulose.

B. J. R.

Propagation by Cuttings.—N. H. Grace ("Effects of Potassium Acid Phosphate, Cane Sugar, Ethyl Mercuric Bromide, and Indolylacetic Acid in a Talc Carrier on the Rooting of Stem Cuttings," *Can. J. Res.*, 1941, **19**, 99–105). Greenwood cuttings of *Deutzia Lemoinei*, *Symphoricarpus albus*, and *Weigelia rosea*, and dormant cuttings of *Lonicera tatarica* were used in this series of experiments. Low concentrations of phosphate tended to increase rooting and reduce mortality of two of the species of greenwood cuttings, whereas the 10 p.c. concentration was ineffective or injurious. However, this concentration was favourable to the rooting of dormant cuttings. Indolylacetic acid treatment increased the number of rooted cuttings and the number and length of roots. Beneficial effects were indicated for organic mercury and cane sugar treatments. However, these were attributed largely to the combinations with phosphate and indolylacetic acid. The results

indicate that the effectiveness of dusts containing indolylacetic acid in the treatment of plant stem cuttings may be increased by the addition of nutrient and disinfectant chemicals. B. J. R.

The Rooting of Herbaceous Cuttings.—N. H. GRACE ("Effects of Talc Dusts containing Phytohormone, Nutrient Salts, and an Organic Mercurial Disinfectant on the Rooting of Herbaceous Cuttings," *Can. J. Res.*, 1941, **19**, 177–82). Cuttings of *Coleus Blumei*, *Chrysanthemum*, and *Iresine* were used for these experiments. Naphthylbutyric acid treatment increased the number of roots per rooted cutting, and its combination with nutrient salts increased fresh root weight of *Coleus* cuttings. Organic mercury treatment increased the number of *Chrysanthemum* cuttings that rooted and the number of roots on *Iresine* cuttings. Beneficial effects from talc treatment alone were a feature of the results. B. J. R.

Wood Structure of Strychnos.—R. A. COCKRELL ("A Comparative Study of the Wood Structure of Several South American Species of *Strychnos*," *Amer. J. Bot.*, 1941, **28**, 32–41, 2 pls.). The wood structure of twenty-six species of *Strychnos* from tropical America is described and figured and their distinctive features indicated. Many of the species are so similar that it has been impossible to formulate any reliable scheme for the identification of their woods. B. J. R.

Wood Structure of American Rutaceæ.—S. J. RECORD and R. W. HESS ("American Woods of the Family Rutaceæ," *Trop. Woods*, 1940, **64**, 1–28). Material of twenty-four out of the forty-four genera represented in the New World have been studied in this investigation. B. J. R.

Wood Structure of American Meliaceæ.—S. J. RECORD ("American Woods of the Mahogany Family," *Trop. Woods*, 1941, **66**, 7–33). The American Meliaceæ are of seven genera. *Elutheria* is presumably very rare, and no mature woody material has been studied. The woods of the other genera are described.
B. J. R.

CRYPTOGAMIA.

Pteridophyta.

Regnellidium.—NOE HIGINBOTHAM ("Development of the Gametophytes and Embryo of *Regnellidium diphyllum*," *Amer. J. of Bot.*, 1941, **28**, 282–300, 105 figs.). *Regnellium*, a monotypic genus of the Marsileaceæ, was first described by C. A. M. Lindman (in *Arkiv för Bot.*, 1904). The development of the male and female gametophytes and of the embryo are now described and figured for the first time, and show that the genus corresponds closely with *Marsilea* and *Pilularia*. The sporocarps, however, do not dehisce with regular valves like those of the other two genera, but simply split and allow the sporangia to escape in a mucilaginous mass. The microsporangium divides internally and forms a male gametophyte, consisting of two small prothallial cells and sixteen spermatids; the latter have two to five coils. The megasporangia also divide internally, first cutting off a large upper cell at the apex (beneath the projecting apical papilla). From divisions of this upper cell is formed the archegonium, consisting of an egg, a neck canal cell, and ventral canal cell, a venter (one or two layers) and a neck (two layers of four cells). The basal part of the megasporangium is occupied by a large prothallial cell. The male and female gametophytes become mature in about one day at a temperature of 68–70° F. The fertilized egg becomes divided first by a vertical wall (parallel with long axis of archegonium); the second wall is also vertical; and

the third is transverse. The young embryo, at that stage, consists of eight cells, two of which will produce the cotyledon; two other cells in the same hemisphere will produce the stem and the second leaf respectively. In the other hemisphere one large cell functions from the first as the tetrahedral root initial, and two others develop into the foot of the embryo. The later leaves have a two-sided apical cell.

A. G.

Pteridium.—R. M. TRYON, JR. ("A Revision of the Genus *Pteridium*," *Rhodora*, 1941, **43**, 1–31, 37–67, 4 pls., 12 maps). A detailed systematic account of *Pteridium aquilinum* and its twelve varieties, with descriptions, critical notes, distribution, and a copious citation of specimens. A key to the varieties is provided.

A. G.

Aleuritopteris.—R. C. CHING ("Studies of Chinese Ferns — xxxi. *Aleuritopteris*." *Hong Kong Naturalist*, 1941, **10**, 194–204). The author revives the generic name *Aleuritopteris* Fée (1852) for a group of ferns which have usually been included in *Cheilanthes* but differ from it in habit, in their broad, flat, entire ultimate segments, in the confluent linear sori, and the broad, flat, brown linear and continuous indusium, and in the naked leaf surfaces, which are usually farinose with white or yellow mealy powder. The type of this group is *Pteris farinosa* Forskal, and most of the species belong to temperate Asia, whereas *Doryopteris*, to which some of the species have been referred by authors, is almost confined to tropical America. A systematic enumeration is given of the species of *Aleuritopteris*, with their synonymy and distribution, and some critical notes. A. G.

Diplazium.—R. E. HOLTTUM ("The Fern Genus *Diplazium* in the Malay Peninsula," *Gardens' Bulletin Straits Settlements*, 1940, **11**, 74–108, 6 figs.). An account of the twenty-five species of *Diplazium* found in the Malay Peninsula, with a key, specific descriptions, and critical notes. It is found that the scales at the base of the stipes are of great help in distinguishing species of similar habit. Four new species are described: *D. Kunstleri*, *D. procumbens*, *D. riparium*, *D. simplicivenium*. A. G.

Rhizoids of Marchantiales.—MARJORIE MCCONAHA ("Ventral Structures effecting Capillarity in the Marchantiales," *Amer. J. of Botany*, 1941, **28**, 301–6, 8 figs.). In 1939 the author [*op. cit.*, **26**, 353–5] demonstrated the importance of the ventral appendages of *Conocephalum conicum* in the external conduction of water along the under side of the thallus. The present paper is a morphological study of the scales and rhizoids of *Preissia quadrata*, *Reboulia hemisphærica*, *Lunularia cruciata*, and *Marchantia polymorpha*, and of the part played by them in external water conduction. The rhizoids are of two kinds: smooth and tubercular. The smooth rhizoids emerge from enclosures beneath the ventral scales, and may make contact with the soil on which the thallus grows; the tuberculate rhizoids originate beneath the scales, regardless of their arrangement, and form numerous and connected capillary strands parallel to the thallus. The efficacy of this capillary system varies with the form and imbrication of the scales and with the length and number of the enclosed rhizoids. A rapid distribution of water to all absorptive areas of the thallus is made possible. A. G.

Bornean Mosses.—H. N. DIXON ("New and Rare Bornean Mosses," *J. of Botany*, 1941, **79**, 57–62, 72–7). A list of forty-five rare mosses found in the Bornean gatherings made by various collectors—Brooks, Clemens, Everett, Hose, Lobb, and others. Ten of the species are new to science, six are new records for Borneo, and two have to be transferred to other genera, their fruit being now known.

The new species belong to the following genera: *Sphagnum, Symblepharis, Dicranoloma, Stephanodictyon, Rhacopilum, Garovaglia, Chaetomitrium, Clastobryum, Warburgiella, Ectropothecium.* A. G.

Thallophyta.

Algæ.

Chrysophaeum.—IVEY F. LEWIS and HILAK F. BRYAN ("A new Protophyte from the Dry Tortugas," *Amer. J. of Bot.*, 1941, **28**, 343–8, 4 pls. and 5 figs.). An account of a new genus and species, *Chrysophaeum Taylori*, belonging to the Chloromonadinæ. It grows on coral and sand in shallow water around the Dry Tortugas, Florida. It forms soft, yellow, flocculent masses (branched colonies of very numerous cells); the cells are stalked, longly pyriform, provided with a pellicle and numerous chloroplasts, but without flagella and stigmata; they exhibit a characteristic motion before reproduction; multiplication is effected by longitudinal fission of vegetative cells, and reproduction by aplanospores which produce zoospores with two equal flagella laterally attached, one of them active, the other trailing. The colonies are 1–6 cm. high; the cells about 80μ long by 30μ wide. The cell-structure and reproduction are described in detail, and illustrated with sixty-three figures. A. G.

Green Snow.—ERZSÉBET KOL ("The Green Snow of Yellowstone National Park," *Amer. J. of Bot.*, 1941, **28**, 185–91, 75 figs. 1 diagram). Green snow was first observed in Spitzbergen in 1838, and has since been recorded in Greenland, on some of the Swiss Alps, on the Tatra Gebirge, and in the Antarctic. In Europe the colour has been found to be due to different species of *Rhaphidonema*. Green snow is now reported from Yellowstone Park, the colour being due to immense quantities of *Chlamydomonas yellowstonensis* Kol, a new species, associated with eight other small algæ and a fungus. A table of all recorded micro-organisms of green snow is provided. Red snow is a much more common phenomenon, and is characteristic of silicotrophic snowfields, whereas green snow is characteristic of calcitrophic snowfields. A. G.

Westiellopsis.—MERCIA JANET ("*Westiellopsis prolifica*, gen. et sp. nov., a New Member of the Stigonemataceæ," *Annals of Botany*, 1941, N.S. **5**, 167–70, 2 figs.). Description and figures of *Westiellopsis*, a new genus found in cultures of soil algæ from the Agri-horticultural Society's Gardens, Madras. It is a blue-green alga, consisting of primary filaments more or less creeping, and producing thinner erect branches, mono-stichous, and without a sheath; heterocysts intercalary; the dilated terminal portions of the branches, by profuse transverse and longitudinal division, form clusters of rounded cells called "pseudohormocysts"; the cell-contents of the latter escape as gonidia and develop into new plants. By the absence of a sheath this genus is distinguished from *Westiella* and *Leptopogon*; its affinity is rather with *Chondrogloea*. A. G.

Dictyosphaerium.—M. O. P. IYENGAR and K. R. RAMANATHAN ("On Sexual Reproduction in a *Dictyosphaerium*," *J. Indian Bot. Soc.*, 1940, **18**, 195–200, 14 figs. and 1 pl.) An account of life-history of a new species of *Dictyosphaerium* (*D. indicum*) from Madras. Sexual reproduction of an oogamous type was found in the plant and is described in detail and figured. The process is rare and occurs towards the end of the vegetative season. The colonies involved are diœcious, some producing bi-ciliate antherozoids, and the others non-motile eggs. A. G.

Gametes in Caulerpa.—M. O. P. Iyengar ("On the Formation of Gametes in *Caulerpa*," *J. Indian Bot. Soc.*, 1940, **18**, 191–4, 9 figs.). Reproduction in *Caulerpa* was first observed by Dostal in 1928; Ernst (1932) found in *C. clavifera* bi-ciliate swarmers of two sizes: Iyengar (1933) found bi-ciliate swarmers in *C. ramosa* var. *uvifera* and observed them conjugating anisogamously; in 1937 Miyake and Kunieda observed anisogamous conjugation in *C. brachypus*. Iyengar now gives fuller details and figures of his observations made in 1933, and claims *C. racemosa* var. *uvifera* to be monœcious, whereas Ernst found *C. clavifera* to be diœcious. A. G.

Indian Charophytes.—S. C. Dixit ("The Charophytes of the Bombay Presidency—II," *J. Indian Bot. Soc.*, 1940, **18**, 231–9, 4 figs.). In two previous papers (*op. cit.*, 1931 and 1935) the author has recorded sixteen species of charophytes from the Bombay Presidency. He now adds four more species, one of which is new to science, namely *Chara Hatei*. Supplementary notes are also given on several of the species recorded in the earlier papers. A. G.

Xiphophora.—Marjorie Mitchell ("Some Studies in the Fucales of New Zealand.—III. Some Investigations on *Xiphophora chondrophylla* (R. Br. ex Turner) Harv.," *J. of Botany*, 1941, **79**, 49–56, 65–71, 4 figs.). Material was obtained from the Bay of Islands, New Zealand. Its morphology and anatomy were investigated, and showed the plant to be intermediate between var. *maxima* and var. *minor* of Agardh. The growing point was found to be fucaceous; secondary filaments were observed arising from the inner layer of the storage region; differences between the sterile and fertile regions were noted. The development of the four oospheres was followed: the megasporangium first produces four tetraspore nuclei; there is then a brief eight-nucleate stage, four supernumerary nuclei degenerating quickly; wall formation occurs *after* this stage. Experiments made on the walls of the oocyte indicate that the mesochiton is three-layered in certain regions and is the prime agent in effecting the escape of the oocyte. The genus is rightly assigned to the Fucaceæ; it is of affinity with other genera which produce four eggs—*Durvillea, Hormosira, Ascophyllum*, and *Bifurcaria laevigata*. A. G.

Algæ of Long Island.—Wm. Randolph Taylor ("Marine Algæ from Long Island," *Torreya*, 1940, **40**, 185–95). A list of sixty-five algæ collected by Dr. Alfred Perlmutter during fishery investigations around the coast of Long Island, New York, where the algal flora is of interest as being intermediate in character between the northerly forms of Cape Cod and the southerly forms of Virginia. A few additions are made to the records, and a much more accurate account of the distribution of the species in Long Island waters is afforded. A. G.

Rare American Algæ.—Wm. Randolph Taylor ("Reappearance of Rare New England Marina Algæ," *Rhodora*, 1941, **43**, 72–4). *Nemastoma Bairdii*, described by Farlow in 1875, was not collected again on the American coast till 1940, when it reappeared in the type locality. Meanwhile it had been found in Europe and studied by Kuckuck, who referred it to the genus *Platoma* in 1912. *Tilopteris Mertensii* was also found again on the same day at the same place after a disappearance of nine years. Notes are added on other species which are rarely to be found, but of which some appear in some quantity at long intervals. A. G.

Lichens.

Studies in the Trypetheliaceæ.—G. T. Johnson ("Contributions to the Study of the Trypetheliaceæ," *Ann. Missouri Bot. Gard.*, 1940, **27**, 1–50, 4 pls.). The Trypetheliaceæ are a family of Pyrenocarpic lichens characterized by the

immersion of the perithecia in a stroma. In an attempt to decide whether this stroma is in any way essentially different from that of certain Pyrenomycetes, the author has made a detailed study of their development and that of the perithecia in the genera *Melanotheca, Trypethelium, Tomasellia, Laurera,* and *Bottaria.* The results of this investigation show that in none of the representatives of these genera examined is there formation of a body strictly comparable to the stroma of the Pyrenomycetes; in *Melanotheca aggregata* the "stroma" is composed chiefly of the bark-cells of the tree upon which the lichen is growing, and for this type the use of Wainio's term "pseudostroma" is recommended. In *Trypethelium pallescens* and *T. eluteriae* the structure consists of a raised pustule with cortical and gonidial layers containing a few scattered and disintegrated bark-cells, and is referred to as a "substroma." *Tomasellia aciculifera* and *Bottaria cruentata* possess a pseudostroma of the *Melanotheca*-type, while *Laurera madreporiformis* and *L. sanguinaria* have substromata. The development of the perithecia in *Trypethelium annulare* was investigated cytologically. The ascogenous hyphæ were found to give rise to multinucleate filaments, the ends of which undergo conversion into asci, the process of "crozier"-formation being somewhat doubtful. The young ascus is uninucleate, but twice-repeated nuclear division soon gives rise to the eight original nuclei of the ascospores, these subsequently becoming three-septate, with one nucleus in each cell. Germination of the spores was observed in *Trypethelium* and *Melanotheca*; in some a slight growth of mycelium could be obtained on artificial media, but this was never vigorous, and no culture survived for longer than six months. It could not be established whether any sexual process takes place in the formation of the fructifications, but well-developed trichogynes were seen in *Melanotheca aggregata* and *M. concatervans.* With regard to the substrate relationships of the group, it is pointed out that although there is always penetration of the living periderm by the hyphæ, one cannot assume that the lichen is on that account parasitic, although the possibility of saprophytism is by no means excluded. A few anatomical observations were also made on several members of the Pyrenulaceæ (species of *Pyrenula, Arthopyrenia,* and *Anthracothecium*), and in some of these a morphology similar to that of the Trypetheliaceæ was found. Positive chemical reactions with potassium hydroxide, calcium hypochlorite, and paraphenylenediamine were obtained in some species of Trypetheliaceæ and Pyrenulaceæ (tabulated list given). The author comes to the conclusion that the Trypetheliaceæ cannot be regarded as a natural family, on account of the widely divergent structure of the stromatic bodies in its various members, and suggests that its genera might be better distributed among the Pyrenulaceæ as terminal members of various series of development.

I. M. L.

New Species of Parmelia.—Y. Asahina ("Lichenologische Notizen (XV)," *J. Jap. Bot.*, 1941, **17**, 71–6, 5 text-figs.). The following new species of *Parmelia* are described: *P. neglecta* (Formosa, Manchuria, Java), *P. simodensis* (Japan), and *P. manshurica* (Manchuria, Japan). A study of the chemical components revealed that *P. neglecta* contains atranorin and a second unidentified lichen acid, possibly caperatic acid; *P. simodensis* atranorin and d-protolichesterinic acid; and *P. manschurica* usninic acid (in the cortex) and lecanoric acid (in the medulla).

I. M. L.

The Genus Nephroma in Japan.—S. Inumaru ("Studia Lichenum Japoniæ. II.," *Acta Phytotaxonom. et Geobotan.*, 1940, **9**, 51–9, 3 text-figs.). The following new Japanese species and varieties of *Nephroma* are published: *N. squamigerum* Inumaru, with n. var. *plumbeum* Inumaru, and *N. parile* f. *fusconigricans* Inumaru. A key is given to all the Japanese species, of which there are eighteen. I. M. L.

Two Japanese Cladoniæ.—Y. ASAHINA ("*Cladonia verticillata* Hoffm. und *Cladonia calycantha* (Del.) Nyl. aus Japan," *J. Jap. Bot.*, 1940, **16**, 462–70, 3 text-figs.). Material of *Cladonia verticillata* from Japan proved on examination to be chemically heterogeneous. Some specimens contain fumarprotocetraric acid and atranorin, and give a distinct yellow reaction with potash; these have already been separated by Wainio as *C. Krempelhuberi*. Others contain fumarprotocetraric acid only, and are hence KHO − or + brownish. Others, again, homosekikic acid and atranorin. Therefore the author has revised the nomenclature of these forms as follows: (1) fumarprotocetraric acid only present (KHO − or + brownish, Pd + red): *C. verticillata* vars. *evoluta* and *cervicornis*; (2) fumarprotocetraric acid and atranorin present (KHO + yellow, Pd + red): *C. verticillata* vars. *subevoluta* Asahina (n. var.), *subsobolifera* Asahina (n. var.), and *sublepidota* Asahina (n. var.); (3) homosekikic acid and atranorin present (KHO + yellow, Pd + yellow): *C. verticillata* subsp. *C. dissimilis* Asahina (n. subsp.). Another chemical type was discovered by the analysis of some specimens from Europe and N. America, these being found to contain psoromic acid, but no new name has been given to them. *Cladonia calycantha* is restricted to the more southerly parts of Japan; it contains fumarprotocetraric acid, but no atranorin. I. M. L.

Japanese Ciliate Parmeliæ.—Y. ASAHINA ("Lichenologische Notizen (XIV)," *J. Jap. Bot.*, 1940, **16**, 592–603, 8 text-figs.). The following species of *Parmelia* occurring in Japan have cilia on the margins of the thallus: *P. cetrata*, *P. reticulata*, *P. trichotera*, *P. Arnoldii*, *P. crinita*, and *P. subcrinita*. These species have been well defined by Du Rietz (1924) in the morphological sense, and the present paper adds valuable data to our knowledge of their chemical composition, which is, briefly, as follows: *P. cetrata*, *P. reticulata*, and *P. subcrinita* have atranorin (in the cortex) and salazic acid (in the medulla), hence cortex KHO + yellow, medulla KHO + yellow then red; *P. trichotera* and *P. crinita* have atranorin (in the cortex) and stictic acid (in the medulla), hence cortex KHO + yellow, medulla KHO + yellow, Pd + ochraceous to red; *P. Arnoldii* has atranorin (in the cortex) and collatolic acid (in the medulla), hence cortex KHO + yellow, medulla KHO −, KHO($CaCl_2O_2$) + reddish, Pd −. Two new forms are described: *P. cetrata* f. *granularis*, and *P. Arnoldii* f. *pallescens*. I. M. L.

Cladonia macroptera in Japan, and the Lichen-substance Pannarin.—Y. ASAHINA ("Lichenologische Notizen (XII)," *J. Jap. Bot.*, 1940, **16**, 401–4, 2 text-figs.). Material of *Cladonia macroptera* Räs. from Japan was found to contain fumarprotocetraric acid, and two new forms of it are now described: f. *ramosa* Asahina and f. *subnuda* Asahina. The second part of the paper deals with the lichen-substance pannarin ($C_{18}H_{15}O_6Cl$), which was isolated from *Pannaria rubiginosa*, *P. coeruleobadia*, *P. lurida*, *P. sublurida*, and *P. fulvescens*; it gives rise to an orange-red reaction of the medulla with paraphenylenediamine similar to that of fumarprotocetraric acid, from which it may be distinguished by microscopic means (crystal structure). I. M. L.

Lichens from Eastern Asia.—M. M. SATÔ ("East Asiatic Lichens (IV)," *J. Jap. Bot.*, 1940, **16**, 495–500, 1 text-fig.). This continuation of the author's studies on lichens from Eastern Asia (mainly Japan) deals with the genera *Haematomma* (five species, two varieties, a key to the Japanese species given) and *Lecanora* sect. *Placodium* (one species, *L. rubina* (Vill.) Ach.). I. M. L.

Chemistry of Lichens.—Y. ASAHINA ("Lichenologische Notizen (XIII)," *J. Jap. Bot.*, 1940, **16**, 517–22, 3 text-figs.). Japanese material of *Cladonia cariosa* was found to contain beside atranorin a second as yet unidentified lichen-acid.

The chemistry of some of the species of *Alectoria* was determined, the results being: *A. jubata* v. *subcana* contains fumarprotocetraric acid (hence KHO —, Pd + red); *A. implexa* v. *cana* contains barbatolic acid, sometimes known as alectoric acid (hence KHO + yellow, Pd + yellow); *A. Zopfii* contains psoromic acid (hence KHO —, Pd + yellow). Zopf described a lichen-substance which he claimed to have isolated from certain *Alectoriæ* as "Bryopogonsäure"; this substance is now shown to be an artefact (monoacetylprotocetraric acid) derived from the breaking down of fumarprotocetraric acid during analysis. I. M. L.

Chemistry of Cladoniæ.—Y. Asahina ("Chemismus der Cladonien unter besonderer Berücksichtigung der japanischen Arten," *J. Jap. Bot.*, 1940, **16**, 709–27, 1 pl., 6 text-figs.). Chemical investigation of material called "*Cladonia chlorophaea*" showed that this species as formerly understood consists of a mixture of chemically distinct strains, to which the author has given specific names. These are: (1) *C. cryptochlorophaea* Asahina, containing a hitherto unknown lichen-acid, cryptochlorophæic acid, and having the reaction KHO($CaCl_2O_2$) + purple-red, with melting-point 166°; (2) *C. merochlorophaea* Asahina, containing another new lichen-acid, merochlorophæic acid, similar to the foregoing, but with melting-point 153°. These two acids can be distinguished by their crystal structure under the microscope. Another chemical segregate from the *C. chlorophaea*-group, *C. Grayi*, was found to contain a third substance, now called by the author grayaninic acid. *C. chlorophaea* (Flk.) Zopf, as now emended by the author, comprises individuals containing fumarprotocetraric acid only. New forms of *C. merochlorophaea* and *C. cryptochlorophaea*, ff. *inactiva* Asahina, denote phases distinguished by the absence of the accessory fumarprotocetraric acid, and conversely a new form, *aberrans* Asahina, is published for those specimens of *C. Grayi* which contain this substance in addition to the grayaninic acid. *C. cryptochlorophaea* and *C. merochlorophaea* are found to possess a wide distribution in Europe, N. America, Manchuria, and Japan. Grayaninic acid was also found in specimens of *C. borbonica* f. *cylindrica* Evans from N. America, whereas the morphologically similar *C. Balfourii* Cromb. is devoid of this substance. I. M. L.

Microchemical Identification of Lichen-acids.—Y. Asahina ("Mikrochemischer Nachweis der Flechtenstoffe. XI. Mitteilung," *J. Jap. Bot.*, 1940, **16**, 185–93, 11 text-figs.). Perlatolinic acid ($C_{25}H_{32}O_7$), originally found in *Parmelia cetrarioides*, is now identified as a constituent of *Cladonia* (*Cladina*) *impexa* Harm.; it is identical with the substance isolated from *C. impexa* f. *erinacea* by Zopf, and called by him "Erinacein." *C.* (*Cladina*) *Evansi* Des Abb., a North American species, was also found to contain perlatolinic acid, together with atranorin (hence the reactions KHO + yellow, Pd —); some specimens from Japan, morphologically indistinguishable from *C. Evansi*, were found to contain, in addition to the perlatolinic acid, usninic acid instead of atranorin (hence KHO —, KHO($CaCl_2O_2$) + yellow, Pd —), and for these a new species is created: *C. pseudoevansi*. The chemistry of *C.* (*Cladina*) *alpestris* was also studied. It had recently been discovered by Des Abbayes (1939) that this species, although usually negative to paraphenylenediamine, might give in certain individuals the reaction Pd — yellow (f. *aberrans* Des Abb.). The present author finds that this yellow reaction in f. *aberrans* is due to the presence of psoromic acid, which is lacking in the typical state of the species. All these lichen-acids can be identified by the author's microchemical method, which is based upon differences in the structure of the crystals obtained from solutions of the substances in glycerin and acetic acid or glycerin, alcohol, and chinolin.

I. M. L.

Fungi.

Woroninaceæ.—D. A. McLarty ("Studies in the family Woroninaceæ—I. Discussion of a new species including a consideration of the genera *Pseudolpidium* and *Olpidiopsis*," *Bull. Torrey Bot. Cl.*, 1941, **68**, 49–67, 26 figs.). An account is given of the new species, *Olpidiopsis Achlyae* which has smooth and spiny-walled zoosporangia and sexual and asexual resting spores. The non-cellulose bristles are apparently formed by localized deposition of host protoplasm upon the zoosporangial surface. The genus *Pseudolpidium* has been merged with *Olpidiopsis* and the diagnosis of the latter raised accordingly. F. L. S.

Chytrids.—C. T. Ingold ("Studies on British Chytrids. I. *Phlyctochytrium proliferum* sp. nov. and *Rhizophidium Lecythii* sp. nov.," *Tr. Br. Myc. Soc.*, 1941, **25**, 41–8, 1 pl., 3 text-figs.). *Phlyctochytrium proliferum*, described as new to science, was found growing parasitically on a species of *Chlamydomonas* in a temporary pond near Reading, while *Rhizophidium Lecythii* n.sp. parasitized the rhizopod, *Lecythium hyalinum*, in water collected from the stream which drains Cropston reservoir in Leicestershire. F. L. S.

Mindeniella.—F. K. Sparrow and V. M. Cutter ("Observations on *Mindeniella spinospora*," *Mycologia*, 1941, **33**, 288–93, 1 fig.). On examining fresh collections of *Mindeniella spinospora* Kanouse the conclusion is reached that this phycomycete undoubtedly belongs to the Leptomitales and not to the Blastocladiales, where its discoverer placed it: among other characteristics, its pedicellate reproductive organs, cellulose walls, and biflagellete zoospores indicate this affinity. F. L. S.

Achlya.—F. T. Wolf ("A New species of *Achlya* from Costa Rica," *Mycologia*, 1941, **33**, 274–8, 13 figs.). The fungus which is named *Achlya Rodrigueziana* sp. nov. was isolated from soil in two localities in Costa Rica. A distinctive feature is the production of but one or two oospores within a small oogonium with generally diclinous antheridia, instead of androgynous antheridie as in *A. orion*, *A. apiculata*, *A. Hähœliana*. F. L. S.

Pythium.—C. Drechsler ("Three species of *Pythium* with proliferous sporangia," *Phytopath*, 1941, **31**, 478–507, 13 figs.). A fungus found on diseased portions of water-lily leaves is described for the first time with the name *Pythium marsipium*. It has generally terminal, asymmetrical utriform zoosporangia of a kind recently considered characteristic for *Pythiogeton*. Proliferous development of sporangia occurs under thoroughly aquatic conditions. Besides this species, *P. oedochilum* and *P. palingenes* are described. They resemble *P. helicoides* in symmetrical conformation and proliferous development of their sporangia, which, however, are rarely corymbose. F. L. S.

Phycomycetes.—C. Drechsler ("Four Phycomycetes Destructive to Nematodes and Rhizopods," *Mycologia*, 1941, **33**, 248–69, 5 figs.). Two fungi, considered from their vegetative morphology and predacious habit to belong to Zoopagaceæ, are described under the new genus, *Cystopage*, proposed for them. This is characterized by the possession of hyaline slightly branched mycelium, which captures minute animals, such as nematodes and rhizopods, and penetrates them, and by reproducing asexually by means of terminal intercalary or lateral chlamydospores, within or upon the substratum. A new species of *Acaulopage*, *A. stenospora*, with long, slender conidia, and *Cochlonema symplocum* n.sp., with an irregularly convoluted thallus, are also described. F. L. S.

Mildew Resistance.—T. W. WHITAKER and D. E. PRYOR ("The Inheritance of Resistance to Powdery Mildew (*Erysiphe Cichoracearum*) in Lettuce," *Phytopath.*, 1941, **31**, 534–40, 2 figs.). *Erysiphe Cichoracearum* was found in *Lactuca scariola* and *L. sativa.* It causes considerable damage to greenhouse lettuce. Strains of lettuce homozygous for susceptibility and for immunity from the mildew were isolated, and subsequent matings and analysis indicated that immunity is controlled by a single dominant gene. F. L. S.

Sclerotinia and Botrytis.—P. H. GREGORY ("Studies on *Sclerotinia* and *Botrytis.* I.," *Tr. Br. Myc. Soc.*, 1941, **25**, 26–40, 3 pls., 1 text-fig.). The connection between *Sclerotinia* and *Botrytis* was demonstrated by exposing material naturally infected with *Botrytis* in the open air, by single-spore cultures of conidia and ascospores and by reinoculation. Connections between conidial and sexual stages were proved for *Sclerotinia polyblastis* Gregory, *S. narcissicola* n.sp., and *S. sphaerosperma* n.sp. F. L. S.

Botrytis and Sclerotinia.—G. B. RAMSEY ("*Botrytis* and *Sclerotinia* as potato tuber pathogens," *Phytopath*, 1941, **31**, 439–48, 2 figs.). A *Botrytis* of the *cinerea* type was isolated from potato tubers from California. Wound inoculation experiments proved this organism to be strongly pathogenic at 40° F. in high humidity. If kept at 70° F., when inoculated with the fungus, for less than three days, infection occurs if then transferred to a temperature of 40° F. If, however, the inoculated tubers are kept steadily at 70° wound periderin is formed and acts as a barrier to fungal invasion. Tubers were also inoculated with *Sclerotinia* species, the perfect stage of *Botrytis* spp. *S. minor* was the most virulent. F. L. S.

New Rust.—E. B. MAINS ("*Tegillum*, A New Genus of the Uredinales," *Bull. Torrey Bot. Cl.*, 1940, **67**, 705–10, 7 figs.). A rust found on leaves of *Vitax* sp. in British Honduras is described as new to science under the name *Tegillum fimbriatum* nov. gen. et sp. The angular uredinospores, and hyaline, one-celled clumped teliospores, both developed in sori bordered by paraphyses, indicate a close affinity with *Olivea* and *Desmotelium*. It differs from them in the position and development of sori from the ganetophytic mycelium. F. L. S.

Physalacria.—G. E. BAKER ("Studies in the Genus *Physalacria*," *Bull. Torrey Bot. Cl.*, 1941, **68**, 265–88, 105 figs.). A detailed study of *Physalacria inflata* and a diagnostic account of the twelve other species so far established for the genus. F. L. S.

Bovistina.—W. H. LONG and D. J. STOUFFER ("Studies in the Gasteromycetes II. *Bovistina*, A New Genus," *Mycologia*, 1941, **33**, 270–3, 1 fig.). Sixty specimens of the gasteromycete described were found in debris under *Juniperus monosperma* in New Mexico. As externally they have the appearance of a *Bovista* while internally the glebal characters of a *Geaster* are exhibited, they are made the type, *B. atrogleba*, of the new genus *Bovistina*. F. L. S.

Helminthosporium.—A. J. ULLSTRUP ("Two Physiologic Races of *Helminthosporium Maydis* in the Corn-Belt," *Phytopath.*, 1941, **31**, 508–21, 6 figs.). An account of two races of a fungus morphologically indistinguishable from *Helminthosporium Maydis* Nisikado and Miyake. Race 1 apparently is confined to the inbred race of corn, "Pr," in which it causes a severe disease, while Race 2 has a wider host range, occurs on inbred 187–2 and a number of proprietary inbred races of maize. Hyphæ of both races penetrate leaves of sugar cane, rice, sorghum, and Sudan grass and produce purplish spots. It is suggested that both may be indigenous to the Corn-Belt, and are apparently spread by infected seed. F. L. S.

Papulaspora.—B. O. Dodge and T. Laskaris ("Papulaspora Gladioli," *Bull. Torrey Bot. Cl.*, 1941, **68**, 289–94, 2 figs.). The so-called smut disease of gladiolus has been shown to be a species of *Papulaspora*. It has been studied cytologically, and an emended description of the new combination, *Papulaspora Gladioli*, is given. F. L. S.

Holly Disease.—E. S. Luttrell ("Tar Spot of American Holly," *Bull. Torrey Bot. Cl.*, 1940, **67**, 692–705, 16 figs.). A cultural and morphological study was made of a tar spot disease of *Ilex opaca* Ait. in North Carolina, caused by the fungus previously known as *Rhytisma Curtisii* B. and Rav. and later *Macroderma Curtisii* (B. and Rav.) v. Hoehn. It is now transferred to the genus *Phacidium*, and its development accords essentially with that of other members of the family Phacidiaceæ. F. L. S.

Tung Oil Tree Disease.—S. H. Ou ("A Study of the *Cercospora* leaf-spot of Tung Oil Tree," *Sinensia*, 1940, **11**, 175–89, 6 figs.). A leaf disease of some economic significance occurred on the Tung oil tree, *Aleurites Fordii* Hemsl. in Chungking. It is caused, apparently, by *Cercospara Aleuritidis* Miyake, previously found on *A. cordata* at Hunan. Conidial stages were found on living leaves and spermogonia and perithecia were found on fallen leaves. Genetical connection between the two stages was proved culturally and the perfect stage is named *Mycosph rella Aleuritidis* n. sp. F. L. S.

Bermuda Fungi.—F. J. Seaver and J. M. Waterston ("Contributions to the Mycoflora of Bermuda—II.," *Mycologia*, 1941, **33**, 310–17, 2 figs.). An account of eight species of *Stictis*, five of which are described for the first time. F. L. S.

British Hyphomycetes.—E. M. Wakefield and G. R. Bisby ("List of Hyphomycetes Recorded for Britain," *Tr. Br. Myc. Soc.*, 1941, **25**, 49–126). This is the third list of British fungi compiled under the auspices of a sub-committee of the British Mycological Society. The Hyphomycetes are here arranged under two main groups: the Gloiosporæ, where the spores are freed from the parent hyphæ by the latter's secretion of moisture or mucilage, the spores being thus frequently held together in a cluster; and the Xerosporæ, which have dry spores, wind dispersed very commonly. F. L. S.

Florida Fungi.—W. A. Murrill ("Some Florida Novelties," *Mycologia*, 1941, **33**, 279–87). Sixteen agarics from Florida are described as new to science and three new combinations are made. F. L. S.

Indian Rusts.—K. C. Mehta ("Further Studies on Cereal Rusts in India," *Imp. Coun. Agr. Res.*, Sci. monogr., No. 14, 1940, 1–224, 12 pls.). The monograph embraces a study of the physiologic races of *Puccinia Graminis Tritici*, *P. Graminis Avenæ*, *P. triticina*, and *P. glumarum* as they occur in India. Work was done in connection with their distribution and prevalence, differential hosts, admixture of races in nature, single-spore cultures and tests for resistance. A special investigation was made on the role of the alternate hosts, *Berberis* and *Thalictrum*, and of over-summering in relation to annual occurrence. F. L. S.

Advance in Mycology.—J. Ramsbottom ("The Expanding Knowledge of Mycology since Linnaeus," *Proc. Linn. Soc.*, London, 1941, **151**, 280–367, 3 pls.). A sketch of mycology since the time of Linnaeus when the subject was in its infancy, the little that was then known about fungi being confined chiefly to their gastronomic effects. In this detailed review many references and historical facts previously overlooked have been brought to light. F. L. S.

NOTICES OF NEW BOOKS.

Cine-Biology.—By J. V. Durden, Mary Field, and F. Percy Smith. 1941. 128 pp., 119 plates, and text-figs. Published by Penguin Books, Ltd., Harmondsworth, Middlesex. Price 6*d.*

It is sometimes a subject for regret by microscopists that cinematograph records of living subjects have not obtained a wider and more appreciative audience. Perhaps the reason for this apparent neglect is to be found in the preface to this little but extremely well conceived book. It is suggested that although the subject is itself of enthralling interest the method of presentation by those learned in science is not only incomprehensible but intolerably dull to the average reader or listener. There is probably only too much truth in this indictment. Many scientists with all the necessary knowledge at their disposal are not able to present their own subject to an audience whose untrained minds cannot appreciate even the simple principles of biological inquiry. This book sets out to repair this shortcoming and admirably it fulfils its purpose. The first unicellular organism dealt with is Amœba, both diagrams and photographs are used for illustrations. This opening chapter is called " The Dawn of Life," not perhaps a title of scientific accuracy but justifiable enough when its purpose is appreciated. To take the reader back to a still earlier stage in the evolution of living things, such as the gene and virus problems present, might be more accurate but would at once introduce a complexity that it would puzzle even some scientists to explain. The development in picture form of the life history of some similar low forms of life is disclosed with success. This indicates the general purpose of this little book and it may be commended to the non-scientific reader with an interest in such matters.

Perhaps in the days to come an educational effort by some scientific societies may be arranged for the general public. Should this come to pass the Royal Microscopical Society might establish for itself a reputation for popular scientific exposition that would be difficult to equal. The subject of microscopy in any of its numerous and expanding applications could present pictorially a story of enthralling interest. J. E. B.

PROCEEDINGS OF THE SOCIETY.

The Council orders for the information of the Fellows the publication of the following summary of the Society's Proceedings since the last report.

New Fellows.—The following have been elected Ordinary Fellows of the Society :—

R. S. Allan, B.Sc.	Worcester Park.
W. O. Barrington, B.Eng.	Shanghai.
J. K. Enock.	Wembley.
H. Gunnery.	London.
H. M. Malies.	Manchester.
M. H. Mannering.	Walthamstow.
D. G. Rowson, B.Sc.	Purley.
G. W. Towell.	Wakefield.
R. J. Trott.	Aston Clinton.
A. V. Weatherhead.	Greenford.

Deaths.—In addition to those recorded in the Annual Report of the Council (see p. 67) the death of the following Fellows is reported :—

H. E. Hurrell.	Elected 1913.
J. Rheinberg.	„ 1899.

Donations.—The following donations have been received and duly acknowledged on behalf of the Fellows :—

Trustees of the British Museum—

“Instructions for Collectors. No. 4A. Insects.” By John Smart.

“Instructions for Collectors. No. 9A. Invertebrate Animals other than Insects.” By Baylis and Munro.

“Ruwenzori Expedition, 1934–5.” Vol. II, Nos. 4 and 5. Vol. III, Nos. 6–10.

“Mosquitoes of the Ethiopian Region. III.—Culicine Adults and Pupæ.” By Edwards. 1941.

Chapman and Hall, Ltd.—

“Handbook of Chemical Microscopy.” By Chamot and Mason. Vol. II. Second Edition. 1940.

Wool Industries Research Association—

“Animal Fibres of Industrial Importance: Their Origin and Identification.”

Comstock Publishing Co., Ltd.—
"The Microscope." By Gage. 17th Edition.

J. E. Barnard, F.R.S., P.R.M.S.—
"Journal of the Photomicrographic Society."
"Les Diatomées." By Pelletan. 1888.

Penguin Books, Ltd.—
"Cine Biology." By Durden, Field and Smith.

Macmillan & Co., Ltd.—
"Plant Science Formulæ." By McLean and Cook.

Chetwynd Palmer, F.R.M.S.—
Travis's original friction attachable mechanical stage, by Swift.

F. D. Armitage, F.R.M.S.—
18 reference slides of Paper-Making Fibres.

Mrs. Redfern—
Small folding French microscope with accessories in case, by Brunner.

Papers.—The following papers received have been considered and approved by Council for publication in the Society's *Journal* :—

J. E. Barnard, F.R.S., P.R.M.S.—
"The Centenary of the Royal Microscopical Society, 1839–1939. Some Personal Recollections and Impressions."

John R. Baker, M.A., D.Sc.—
"A Fluid for Softening Tissues Embedded in Paraffin Wax."

Prof. H. Graham Cannon, Sc.D., F.R.S.—
"On Chlorazol Black E and some other New Stains."

P. N. Bhaduri, M.Sc., Ph.D., F.R.M.S., and C. S. Semmens, F.R.M.S.—
"Nucleolar Staining Method Applied to Animal Tissues."

Manfred Voigt, F.R.M.S.—
"Contribution to the Knowledge of the Diatom Genus *Mastogloia*."

102ND ANNUAL REPORT.

REPORT OF THE COUNCIL FOR THE YEAR 1941.

The past year has been an eventful and anxious one for those entrusted with the conduct and maintenance of the Society's activities and the custody of its Library and Historical Collections under the conditions prevailing.

During the frequent and heavy enemy air raids on London the Secretary received numerous intimations of damage and destruction affecting the executive work of the Society. Early in the year advice was received of the total destruction of premises of the Society's blockmakers, while in a subsequent raid the premises of the Society's printers were destroyed and all stock and matter then in hand for

publication irretrievably lost. In later raids a whole series of scientific abstracts prepared for publication in the *Journal* was destroyed, and subsequent information received from the accountants advised that the whole of the Society's books of accounts and records which were in their possession for audit had been totally destroyed by enemy action. Nor did the Society escape the effects of high-explosive and incendiary bombs at and adjoining its premises, though it is gratifying to report that the Library and collections suffered only minor damage.

Notwithstanding these and many other embarrassing difficulties the essential activities of the Society have continued to be carried on without intermission, and the work of the office, library, reference collections, and publication have been maintained throughout. Numerous requests have been received for information and advice on matters concerning technical and applied microscopy in scientific and industrial processes. It will be evident that the task of conducting these under such circumstances has been one of unremitting care and devotion. The heavy additional strain imposed upon the Secretary's office has been considerable and it is regretted that it has frequently not been found possible to deal with the increased correspondence, and with numerous inquiries and requests, with the usual promptitude.

PRESIDENT,

OFFICERS AND MEMBERS OF COUNCIL.

In accordance with Bye-Laws 41–42, and the authority granted by the Lord President of the Council (1941. *Journ. R. Micr. Soc.*, LXI. 73) the following continue in office respectively :—

PRESIDENT,

J. E. Barnard, F.R.S.

VICE-PRESIDENTS,

Dr. R. S. Clay.
M. T. Denne, *O.B.E.*
Dr. G. M. Findlay, *C.B.E.*
D. J. Scourfield, *I.S.O.*

HON. TREASURER,

C. F. Hill.

HON. SECRETARY,

Dr. G. M. Findlay, *C.B.E.*

ORDINARY MEMBERS OF COUNCIL,

C. Beck, *C.B.E.*
R. J. Bracey.
Dr. H. M. Carleton.
Prof. R. R. Gates, F.R.S.
N. Ingram Hendey.
Dr. R. J. Ludford.
Dr. J. E. McCartney.
Dr. J. A. Murray, F.R.S.
J. H. Pledge.
T. E. Wallis.
H. Wrighton.
S. R. Wycherley.

Hon. Editor, Dr. G. M. Findlay, *C.B.E.*; *Hon. Curator of Instruments*, Mr. M. T. Denne, *O.B.E.*; *Hon. Curator of Slides*, Mr. N. Ingram Hendey; *Secretary and Librarian*, Dr. C. Tierney.

FELLOWS.

The Council deplores the loss the Society has sustained by death of the following Fellows :—

Col. G. C. K. Clowes.	Elected 1935.
A. E. Harris.	,, 1928.
Isaac Hartley.	,, 1925.
H. Lloyd Hind.	,, 1929.
Otto Langer.	,, 1932.
G. H. Nall.	,, 1914.
A. Norman.	,, 1899.
C. H. Oakden.	,, 1920.
H. Rhodes.	,, 1928.
C. H. Wright.	,, 1889.

Three Fellows have been reinstated to the Roll of Fellowship and fifteen candidates have been elected Ordinary Fellows of the Society. Fifteen Fellows have resigned.

MEETINGS.

In view of the increasing number of Fellows now serving with His Majesty's Forces or otherwise fully engaged on work of national urgency adequate attendances at Ordinary Meetings under prevailing circumstances has not been considered practicable and none has been convened. During this temporary curtailment all action required by the By-Laws to be taken at such meetings continues to be discharged by the Council through its appropriate Committees and Executive Officers under the Authority previously mentioned.

JOURNAL.

In response to the urgent request of the Government for the utmost economy in regard to supplies of paper, metals, and other essentials used in printing and illustrating, the volume of the Society's *Journal* for the year has been condensed into two issues containing original papers together with the series of current abstracts of available Empire and foreign scientific publications.

In view of the restrictions mentioned and the very heavy additional costs of printing and illustrating in which the Society finds itself involved, authors submitting original papers for publication are urged to be as succinct as possible compatible with adequate description in cases of new technique.

Fellows of the Society and subscribers will not be unaware of the problems and circumstances under which scientific publication has to be conducted at the present time, and on behalf of the Fellows the Council records with deep appreciation the thanks due to the Society's Honorary Editor, and to the panel of Abstractors, for their valued work in exceptionally difficult conditions.

LIBRARY, INSTRUMENT, AND SLIDE COLLECTIONS.

During the period of intensive bombing the Executive Committee of the Council received and considered a memorandum submitted by the Secretary, with the assent of the Hon. Curator, calling attention to the hazardous risk involved in retaining the whole of the Society's Collection of Historical Instruments in one place in the target area; and asking for authority to pack and distribute—so far as is possible—portions of the collection in other areas for temporary storage and safe custody: it being understood that no liability attaches to the generous friends who offered accommodation, and in some cases transport, for any loss or damage subsequently casued thereto through enemy action.

While it is recognized that nothing that the Council is able to do can ensure the avoidance of risk, this arrangement appeared to be the most satisfactory, and authority was thereupon granted. A portion of the instrument collection has accordingly been packed and distributed for safe preservation during the emergency. A register has been kept of these depositions and the Council takes this opportunity of placing on record its grateful acknowledgment of the generous support of those friends who have rendered the Society this outstanding service.

Increasing use continues to be made of the Society's Library especially with regard to technical and scientific inquiries in connection with the war emergency. In addition to library volumes, certain slides and equipment have been lent to official institutions in response to urgent requests.

Twelve volumes have been added to the Library, and the thanks of the Fellows have been conveyed to the donors.

The thanks of the Fellows have also been conveyed to the donors of two small instruments and eighteen reference slides.

GENERAL.

The Council appointed Dr. Vida A. Latham and Mr. Robert Ross to represent the Society on the occasion of the Fiftieth Anniversary of the foundation of the University of Chicago.

JOURNAL

OF THE

ROYAL MICROSCOPICAL SOCIETY.

SEPTEMBER & DECEMBER, 1942.

TRANSACTIONS OF THE SOCIETY.

V.—THE STRUCTURE AND DEPOSITION OF THE SHELL OF *TELLINA TENUIS*. **594.**

By E. R. Trueman, B.Sc.

(From the Dept. of Zoology, University of Glasgow.)

One Plate, Thirteen Text-Figures.

General Introduction.

Work on shell structure and shell deposition among the molluscs has in general been of two types. Some authors have dealt with the microscopic structure of various shells, while others have discussed chiefly the histology of the mantle and the physiology of secretion of the calcium carbonate. Sufficient work on shell structure has been carried out to establish the fact that comparable microscopic features are present in certain shell layers of a wide range of families, and the characters of the layers found in many genera have been described. Few accounts have been given, however, of the relations of the structural features in the various layers or during growth of the shell of a single species. This is attempted in the present account of *Tellina tenuis*, a species in which the shell structure does not appear to have been previously described.

Attention has also been paid to the structure of the mantle, and to the distribution of calcium within it. A consideration of these factors in relation to the details of shell structure leads to conclusions which are relevant to the general problem of shell formation.

The material used in this work was collected at Millport, Firth of Clyde; part of the work was carried out at the Laboratory of the Scottish Marine

Biological Association, Millport, and part in the Zoology Department of the University of Glasgow. The writer is indebted to Professor E. Hindle, F.R.S., and Mr. R. Elmhirst for facilities and for much assistance. He also wishes to express his gratitude to Mr. A. Ferguson of the Geological Department at Glasgow for expert help in the preparation of sections, and to Professor J. W. Cook, F.R.S., and Dr. S. T. R. S. Mitchell for facilities for the spectroscopic examination of some of the shells.*

Historical Résumé of Work on Structure of the Bivalve Shell.

The first important work on the structure of bivalve shells was that of Hatchett (1799), who divided them into porcellanous and nacreous types. An important advance was marked by Carpenter's famous papers on the subject (1844, 1847); he described various structural types, and asserted (1844, p. 15) that there was considerable uniformity in structure in the shells of any family.

Rose (1858) and Sorby (1879) paid attention to the mineral composition of the shells, showing that some are of calcite, others of aragonite, while a certain number have an outer layer of calcite and an inner layer of aragonite. The chemical composition of many shells was established by Clarke and Wheeler (1917), who showed that varying amounts of magnesium carbonate may be present in calcite shells but that magnesium is absent from aragonite shells.

A good general summary of work on shell structure was given by Haas (1931), while attention may also be drawn to a recent paper by Schenck (1934.) Mention must here be made of the work of Bøggild (1930) who brought together details of the structures of a great range of molluscs and gave a critical account of the main types of microscopic structures met with in the various groups. In the literature of shell structure many different terms are employed for the distinctive arrangements of the crystalline material, but Bøggild's careful description of their crystallographic orientation and structural characters appears to the writer to form a most convenient basis for comparative studies, although modification and extension may be required in certain cases. Bøggild's terminology is adopted in the description of *Tellina tenuis* which follows. For the bivalves at least this terminology has advantages over those proposed by other recent workers, for example Schmidt (1921, 1922).

The Shell Structure of *Tellina Tenuis*.

Introduction.

The first description of the shell of any of the Tellinidæ was given by Carpenter (1847, p. 102); he gave two figures which show crossed lamellar structure. Bøggild (1930) gave a brief account of two modern species, *Macrotoma (Tellina) baltica* and *Tellina calcaria*, and of several Tertiary forms. No account has yet been given of *Tellina tenuis*.

* The author gratefully acknowledges a grant from the Carnegie Trust toward the cost of illustrating this paper.

Methods Used.

Thin sections of shells were made in directions (1) approximately parallel to the surface (referred to below as horizontal sections), (2) normal to the shell and from umbo to margin (radial sections), and (3) normal to the shell and tangential to the growth lines (concrescent sections): these directions are shown in fig. 1, where other descriptive terms used are indicated. In the preparation of thin sections the chief difficulty arose owing to the delicacy of the shell. The following methods gave good results.

In the preparation of horizontal sections, canada balsam was heated on a slide until it became brittle very rapidly on cooling; the shell to be sectioned was then placed on this and the balsam allowed to cool. For sections of the

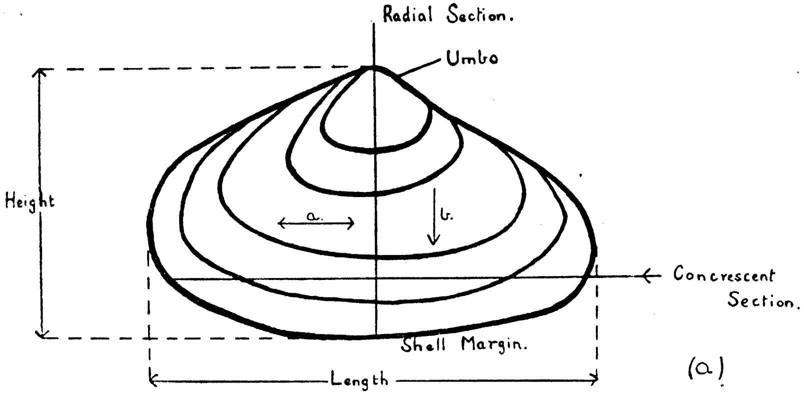

Fig. 1.—Diagram to explain terms used in description of sections. *a*, longitudinal (concrescent) direction; *b*, radial direction.

outer layers the shell was cemented with the inner layers uppermost, and conversely for sections of inner layers. Dilute acid was then used to dissolve away the upper part of the shell, and, after washing, the shell was rubbed very gently with a glass rod and fine carborundum. In this way thin sections of all the layers were obtained; although in most cases the grinding was not uniform throughout the section, sufficient areas for examination were available.

The preparation of vertical sections entailed a different procedure. The shell was placed in plaster of paris or in ordinary cement, which was allowed to set. This was hardened by immersion in canada balsam or bakelite; the air was withdrawn by means of a vacuum pump from the pores and balsam or bakelite replaced it, producing a hard mass. The block was then treated in the usual manner of rock sections, by grinding and then polishing.

The complete interpretation of shell structure is only possible when radial, concrescent, and horizontal sections (the latter in each layer) are available for study.

Mineral Character of the Shell.

Apart from the periostracum, the shell of *T. tenuis* consists wholly of aragonite. This was confirmed by various tests, both chemical and physical.

For most purposes the specific gravity affords a suitable means of distinction ; calcite has a specific gravity of about 2·7 and aragonite about 2·9. The shell to be tested was first boiled in strong potassium hydroxide, to remove all organic material, including the periostracum. After washing and drying, fragments, or, in some cases, a powder of the shell, were placed in a test tube in which a density diffusion column had been made by adding gently a few drops of carbon tetrachloride to bromoform (S.G. about 2·9). The addition of small pieces of mineral calcite and aragonite, which were suspended at their appropriate levels, made it possible at a glance to estimate the specific gravity of any shell fragments introduced.

In thin sections and in microscopic fragments the refractive index proved of great assistance : the most useful point is that in one direction the refractive index of calcite is lower than that of canada balsam, while aragonite never shows an edge below canada balsam. Manigault (1939, p. 353) has lately said that the refractive index is not an easy character for differentiating these forms of calcium carbonate in shells, but, in my experience, by using this character, distinction can be rapidly made.

Crystallographic differences are related to the fact that calcite is a trigonal mineral, while aragonite is orthorhombic : calcite is thus uniaxial, while aragonite is biaxial. With crossed Nicols and convergent light the interference figures given by suitably orientated crystals show characteristic features. The aragonite of many shells has optical characters somewhat different from those of the normal mineral, and Kelly (1901) proposed to regard the aragonite of shells as a special mineral under the name of "conchite." This view has not been adopted, but lately Bøggild has given details of the wide range of variation found in shell aragonite ; the refractive indices are different, and the optic axial angle is often much more acute.

Periostracum.

The shell consists of three layers and the periostracum, viz. :—

Periostracum.
Outer layer.
Middle layer.
Inner layer.

The structure of these layers is discussed in the order given, and is shown in a composite diagram (fig. 2).

The periostracum consists of a very thin layer of conchyolin, probably not more than 0·003–0·004 mm. in thickness in the adult shell. It appears structureless and extends uniformly over the whole shell. In some shells (e.g. *Anodonta*) the periostracum shows the pattern of the prisms below, but this is not found in *Tellina*. When the shell is dissolved away in dilute hydrochloric

acid the periostracum and ligament remain ; the periostracum appears continuous with part of the ligament and remains attached. No trace of struc-

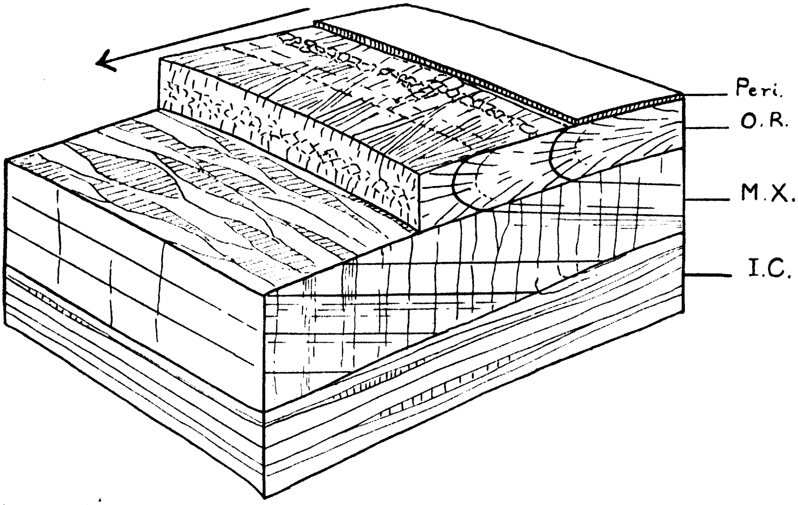

Fig. 2.—Diagrammatic representation of a piece of the shell of *T. tenuis*. Shows periostracum (Peri.), outer radial prismatic layer (O.R.), middle layer of crossed lamellæ (M.X.), and inner complex layer (I.C.) with normal features, in three dimensions.

ture can be seen in the periostracum. The relations of the hinge to the periostracum may be conveniently left until later when the layers of aragonite have been fully discussed (p. 79).

Outer Layer (Composite Prismatic Structure).

The outer layer is seen in horizontal section to be composed of a series of elongated prisms which are essentially radial.

The typical radial prismatic structure runs across the concrescent banding characteristic of this species. This may be clearly seen in pl. I, fig. 1. The radial prisms vary in length ; some are as long as 0·28 mm., but mostly they are shorter, being in the order of 0·15 mm. long. It may be observed that many of these are considerably longer than the distance between the finer concrescent bands (about 0·05 mm.). These long fibres show crystallographic continuity throughout their length. Each fibre has a width of about 0·005 mm. and is in fact a single orthorhombic crystal with its long axis arranged radially. All these parallel fibres thus behave similarly under crossed Nicols.

In some places the fibres exhibit what may be called fanning or feathering. This consists of a series of closely associated crystals being arranged as a slightly divergent bundle (fig. 3).

In addition to the radial prisms there occurs in some horizontal sections of this layer a coarser prismatic structure. This consists of much larger prisms

which are somewhat irregular and appear to form a mosaic in horizontal section. With crossed Nicols these coarser prisms appear more brightly coloured than the radial prisms. This structure occurs only in the outer

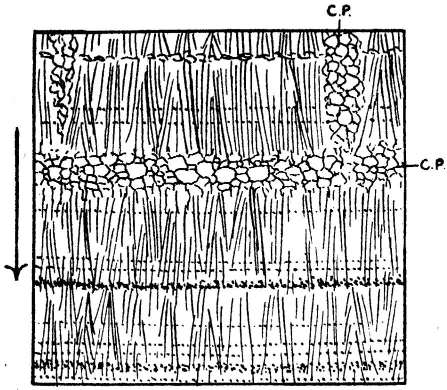

Fig. 3.—A portion of outer layer in horizontal section. Prisms of radial prismatic structure with concrescent bands. C.P., coarse prismatic structure (× 200, approx.).

layer, chiefly in narrow concentric bands alternating with the radial prismatic zones but also occasionally in the form of long irregular wedges interspersed amongst the radial prismatic structures (fig. 3).

In radial sections of the outer layer it is seen that the middle part of the

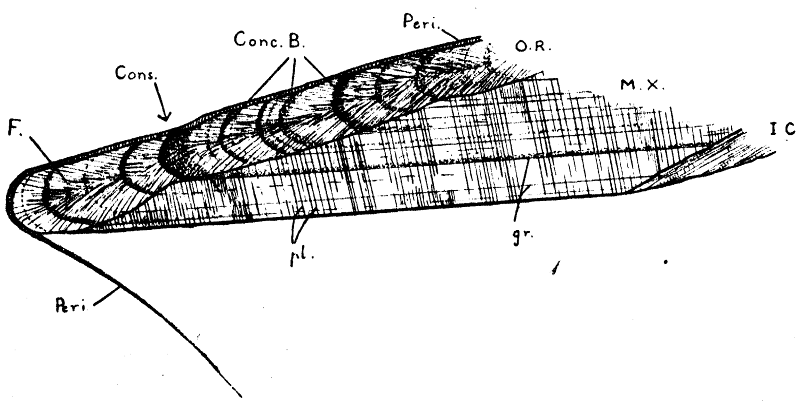

Fig. 4.—Margin of the shell of *T. tenuis* in vertical radial section (× 30). The normal features of this section are shown. Note the loose end of periostracum around the margin of the shell. Cons. constriction. (Other lettering as in figs. 2 and 3.)

layer is composed of horizontal prisms. These appear to fan both inwards and outwards, making an angle of around 30° with the boundary of the layer (fig. 4). On this pattern of fanned-out prisms is superimposed a structure related to their growth, consisting (in radial section) of curved, almost semi-

circular lines of growth: these are similar in shape to the edge of the outer layer at the tip of the shell.

The fanning out of prisms seen in this layer (and characteristic of corresponding layers in many bivalves) resembles the " sphärokristall " structure described by Schmidt in *Nucula* (1922, p. 179), although it is less completely developed. Similar spherulitic structures in the skeleton of Hexacorals have recently been discussed by W. H. Bryan and Dorothy Hill (1941).

In radial section the bands of coarser prisms are not always so distinct, but they are marked by the greater size of the crystals and by the indefiniteness of the radial structure.

The characteristic banding of the shell of *T. tenuis* is a feature of the outer prismatic layer. The bands can be examined in horizontal and radial sections of the layer, but these should not be ground so thin as those for the study of other structures; the bands depend chiefly on the relative opacity of parts of this layer, and in very thin sections they are not very distinct. The bands seen with the naked eye are formed by the grouping together of a large number of thinner bands: the narrowest recognizable on the shell surface are about 0·004–0·008 mm., but these are often grouped in zones of 0·1–0·2 mm. wide. In horizontal sections it is seen that individual radial prisms frequently extend across several of these bands.

In radial section it is seen that these bands are closely related to the curved growth lines in the outer layer (see figs. 2, 4). Marked increase in thickness of this shell layer frequently follows a zone with much opaque material. It is suggested that this latter corresponds with the end of a growth stage, or with a period of slow growth.

Middle Layer (Crossed Lamellar Structure).

The middle layer is composed entirely of aragonite in the form called by Bøggild " crossed lamellar structure " (" Bandförmige Prismen " of Schmidt, 1921 *b*, p. 224). This very specialized structure is found in many gastropods and lamellibranchs, but is never found outside the mollusca. Commonly it is of aragonite, but the corresponding calcite structure is recorded by Bøggild among the Ostreidæ and the Aviculidæ. In the crossed lamellar layer the aragonite is in the form of plates or lamellæ (named first-order lamels by Bøggild, 1930, p. 251) which have their longer directions parallel to the growing edge of the shell. Each lamella (or first-order lamel) is composed of aragonite in optical continuity, but smaller units can be recognized (second-order lamels of Bøggild) within it. These smaller units are not very readily seen in the thin shell of *T. tenuis*, as they are in crystal continuity. The crystal structure, however, is differently orientated in alternate lamellæ (first-order lamels). Accordingly, they extinguish differently with crossed Nicols; in a limited field the lamellæ form two sets, half extinguishing in one position, alternating lamellæ (forming the remainder) in another position, each extinction being inclined at a similar angle to the shell border. The structure thus resembles repeated twinning.

In some molluscs the lamellæ have considerable extent, but in *T. tenuis*, as in many bivalves, they commonly taper at either end, and are comparatively short and sometimes branched, giving a more or less irregular rhomboidal pattern with crossed Nicols (pl. I, fig. 2).

This structure may be examined in both horizontal and radial sections. In horizontal sections the characteristic rhomboidal pattern is commonly seen. The lamellæ are usually about 0·035 mm. long (in the concrescent direction) and 0·008 mm. wide. In *T. calcaria* the lamellæ are coarser, being about 0·02 mm. wide, but in that species the layer is much thicker than in *T. tenuis*. While alternate lamellæ in a restricted field extinguish simultaneously, when a large area of the shell is considered it is seen that the structure is curved with the growth lines.

With crossed Nicols and convergent light some (but not all) horizontal sections of this structure give a good interference figure, suggesting that the crystal axes are arranged normally to the shell surface. The figure obtained shows isogyres which sometimes form an almost complete cross, but, on turning the stage, may show very slight asymmetry. The effect probably results from the symmetrical arrangement of the two sets of lamellæ. It is interesting to note that when the slide is moved about bringing other lamellæ into view, the interference figure is unchanged.

In radial sections the crossed lamellar layer appears as a series of fine plates inclined at an angle of about 85° to the boundaries of the layer (fig. 4). Crossing these lamels approximately at right angles are fine lines, which near the margin of the shell are seen to be parallel to the edge of the crossed lamellar layer at the growing tip, where it is bevelled by the plane of growth (figs. 2, 4). These lines mark successive additions to the layer, and their relation to the curved growth lines in the outer layer is obvious. In some cases there is a distinct opacity in the middle layer along a plane corresponding with an opaque stage in the outer layer (fig. 4).

It must be noted that these growth planes in the middle layer cut across the crossed lamellar structure, but that the crystals show optical continuity throughout the depth of the lamella.

Inner Layer (Homogeneous and Complex Structure).

The inner layer consists of a series of thin sub-layers (averaging for the most part about 0·003 mm. thick) which are most clearly seen in radial section (fig. 4). In some parts it is seen that the sub-layers are of two types, one finely prismatic and tending to become homogeneous (Bøggild, 1930, p. 245), and showing uniform extinction over wide areas between crossed Nicols; the other similar in character to complex crossed lamellar structure (Bøggild, 1930, p. 254). This latter type of structure is very finely developed, and if seen in horizontal section the lamellæ are extremely irregular, the structure persisting for only short distances: small patches in this layer thus resemble that figured by Bøggild for the complex crossed lamellar layer of

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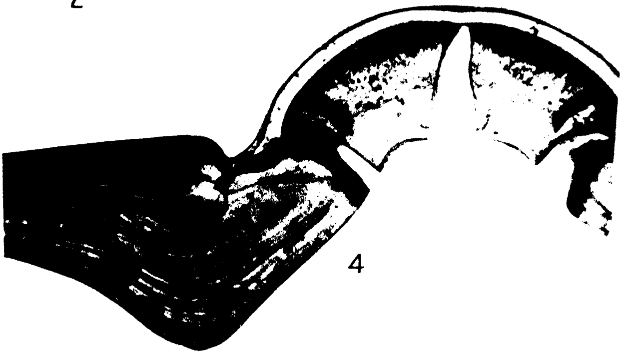

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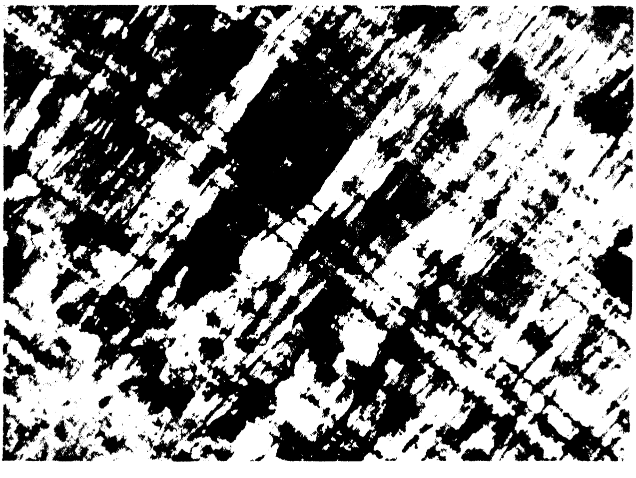

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Lucina sp. (1930, pl. V, 5). The inner layer corresponds to the porcellanous layer of some authors (e.g. Haas, 1931, p. 237).

When the inner layer is thickened, in the umbonal part of the shell, the homogeneous structure is well developed; this extends into the calcareous teeth forming the hinge, and into the nymphæ which support the ligament. The sub-layers of the inner layer expand greatly in thickness as they are traced in this region. The crystals are arranged so that their vertical axes are normal to the surfaces of the sub-layers.

Growth Features in the Shell Layers.

By examination of a vertical radial section it is obvious that the layers of the shell do not remain in the same ratio of thicknesses from the umbo to

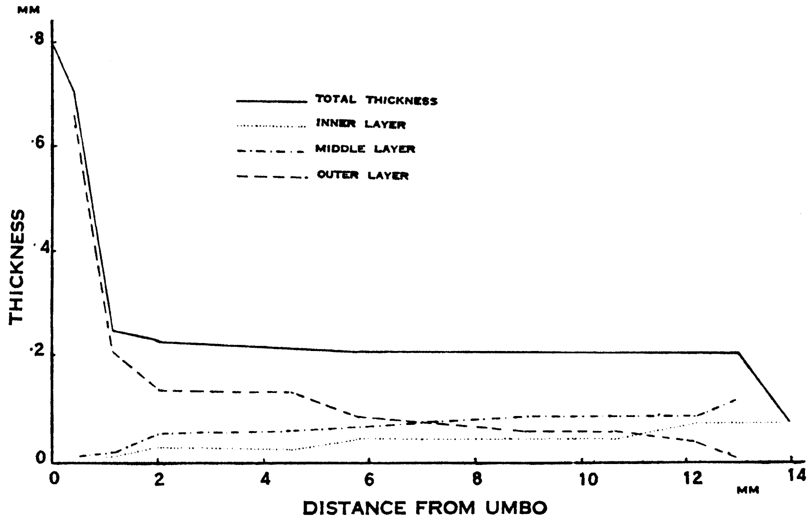

Fig. 5.—Graph of thicknesses of shell layers plotted against distance from umbo for a specimen of *T. tenuis* 14 mm. high.

the margin of the shell. Measurements have therefore been made of several vertical radial sections to investigate more exactly the ratios of thickness between the three layers. From these results graphs have been drawn (figs. 5, 6). Comparable studies appear to be lacking for other shells (except for *Anodonta,* Moynier de Villepoix, 1892, p. 481), but *T. tenuis* is a favourable species for these measurements, since the layers are generally more distinct than in many shells.

The total thickness of the shell increases from the distal edge of the shell to the umbo. This increment is first rapid up to a thickness of around 0·2 mm.; this remains fairly constant for some millimetres, but a rapid increase is made to the maximum thickness in the hinge region. The " mean " thickness (of about 0·2 mm.) is reached in perhaps 1 mm. from the edge of the shell,

whether the shell is large or small. At this point all three layers are generally present. Thus the greater part of the shell is composed of three layers which are together 0·2 mm. thick. This thickness does not vary very much between shells of 3 mm. breadth and much larger specimens of 15 mm. breadth. The larger shells are slightly thicker (usually just over 0·2 mm. in this region) and the smaller ones a little thinner, but not below 0·15 mm. There is thus some relation between thickness and size of shell, but the increase in thickness is not proportionate to increase in size of the shell. Near the umbo thickness increases greatly and rapidly. In a few millimetres the thickness may, in small shells, increase from 0·15 mm. to 0·3 mm., in larger shells from 0·18 mm. to 0·5 mm., and in the largest shells from 0·24 mm. to 0·8 mm. The variations in total thickness are shown in figs. 5, 6.

The outer layer of radial prismatic structure is exceptionally thin at the umbo and increases to the margin, where it composes the major part of the shell. The actual thickness of this layer at the margin thus depends on the

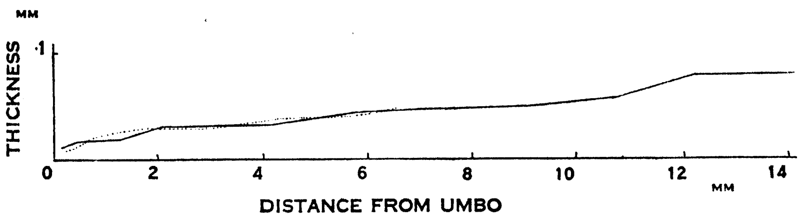

Fig. 6.—Graph of the thickness of outer layer of *T. tenuis* in a large and a small specimen showing similarity of thickness.

size of the shell. If a comparison is made with the thickness of this layer in smaller and larger shells at equal distances from the umbo it is found that the thicknesses are very similar (fig. 6).

When the thickness of the outer layer is plotted graphically against distance from umbo it is seen that the increase is not gradual but is marked by small steps (fig. 5); these may be related to the seasonal growth stages. The thickness of the layer may increase rapidly to a new size on recommencing growth in the spring. In this connection it should be mentioned here that in the radial sections of some shells constrictions occur in the outer layer (fig. 4). These may be correlated with growth pauses (possibly some are seasonal). In a shell obtained in May, expansion of the outer layer follows a fairly recent constriction. Such constrictions occur in one example at the following distances (mm.) from the umbo: 7·4, 10·2, 11·5, 12·7, 13·6, and 13·75; these are probably too frequent to be seasonal (compare Stephen, 1938). There is the possibility of spawning having a similar effect on the shell.

The middle layer (of crossed lamellar structure) shows features in thickness changes which are similar to those of the outer layer. It exhibits, though perhaps not so clearly, the similarity in thickness of the layer in shells of various sizes at the same distance from the umbo. This layer, however, is generally thicker than the outer layer over the whole of its range. At the

distal margin of the shell this layer does not extend to the tip but is "bevelled" along a growth plane at an angle of about 15° to the shell surface (fig. 4).

The innermost layer, when its thickness is plotted graphically, is seen to follow very closely the line of the total thickness. The inner layer thus comprises a large proportion of the shell, the proportion increasing slightly in larger shells: for example, in a small shell the inner layer makes up about 48% and in a large one about 55%. The shell layers may be considered as forming two fundamental units: (*a*) the outer and middle layers, which are formed by the mantle edge and each part of which is not subsequently increased in thickness, and (*b*) the inner layer, formed essentially by the outer surface of the mantle, and regularly increased during growth by subsequent sub-layers. The inner layer is not always quite sharply separated from the middle layer, however, for its structures may be continuous with the lamellæ of that layer. This grouping of the layers may perhaps be compared with the grouping into "ostracum" and "hypostracum" by some authors (Thiele, 1893; Prashad, 1928). Schenck (1934, p. 16) emphasized the need for a correlation of these terminologies, and it appears to be possible by an extension of studies of thickness changes.

Hinge Structure.

The ligament in *T. tenuis* is of a simple character. Essentially it resembles a hollow thick-walled cylinder, split along one side and with part of

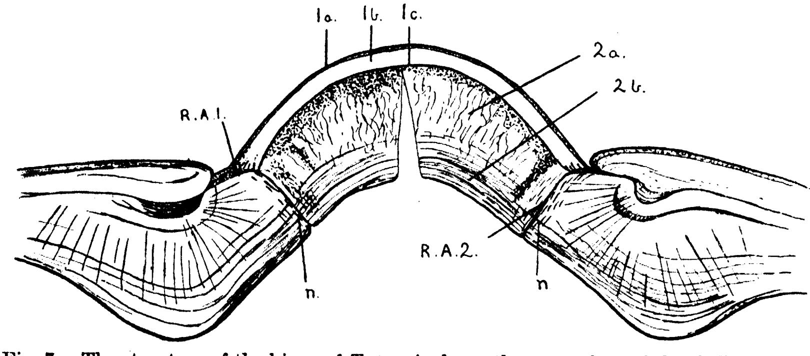

Fig. 7.—The structure of the hinge of *T. tenuis* shows the two valves of the shell connected by ligament (slightly broken) (×80). n. nymphæ; R.A.1 attachment surface of external part of the ligament; R.A.2 attachment surface of internal part of the ligament. 1*a*, 1*b*, and 1*c* refer to the three layers of the external part of ligament and 2*a* and 2*b* to the two layers of internal part (see also text).

the free edges so produced attached to a flat supporting surface along the edge of each valve: these surfaces are called the *nymphæ* (fig. 7). The ligament may thus be described as *parivincular*. Situated wholly behind the umbo, it is said to be *opisthodetic*.

The ligament consists of two parts, the external (1) and the internal (2)

parts (pl. I, fig. 4; figs. 7, 8). It is about 0·3 mm. thick and all layers show strong double refraction in polarized light. In section it closely resembles the ligament of *Tellina* (*Macrotoma*) *baltica* (see Ehrenbaum, 1884, Tab. I, fig. 10; Haas, 1931, fig. 129).

The external part of the ligament is about 0·045 mm. thick and can be further subdivided into three layers. The outer layer (1*a*) is about 0·01 mm. thick and is built up of several (four or five) thin layers. These layers may not continue over the whole of the hinge but extend for long distances. They

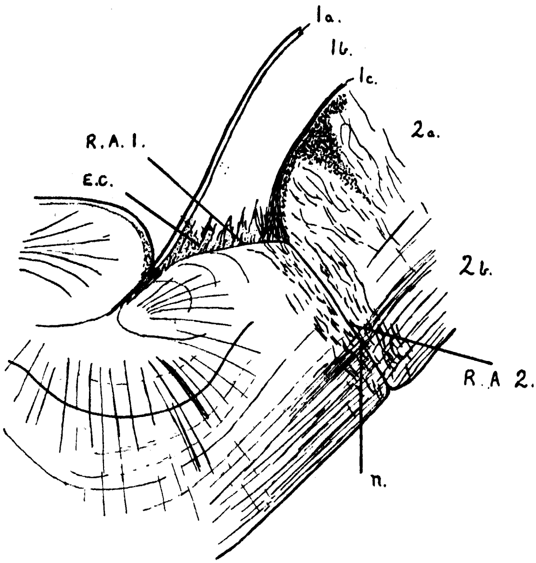

Fig. 8.—Enlarged drawing of the attachment of the ligament to the shell. E.C. elongate crystals at the junction of the external part of the ligament to the shell. (Other letters as in fig. 7.)

are most easily visible with crossed Nicols because they give different colour bands, but are also recognizable in ordinary light.

The middle layer (1*b*) of the external part is approximately 0·03 mm. thick and appears clear, pale yellow coloured, and structureless in ordinary light. With crossed Nicols it gives bright colours and straight extinction in directions normal to the outer surface. No structure was observed with crossed Nicols. The innermost layer (1*c*) of the external part is even thinner than the outer layer, to which it is very similar.

This external part of the ligament is attached to the shell along a plane separate from that of the nymphæ. In the region of attachment this layer is seen to include crystals, elongated along the layer (fig. 8); the external part of the ligament extends beyond the internal part, the end being situated in a deep groove between successive layers of the shell.

No sections were obtained which showed the continuity of the external

part of the ligament with the periostracum. Some continuity is indicated, since when the shell is dissolved away in dilute hydrochloric acid the ligament remains attached to the periostracum. It is probable, however, that the area of contact is very small. Unlike the ligament, the periostracum is not doubly refracting. This may indicate some difference in composition, but the double refraction may be related to strain in the ligament.

The internal part (2) of the ligament (resilium of some authors) is about 0·25 mm. thick in specimens of about 10–12 mm. height. It is more varied in structure and paler in colour than the external part with which it makes a sharp junction. This part of the ligament (unlike the external part) is pleochroic.

The internal layer may be roughly subdivided into two zones which tend to merge into each other. The outermost portion (2*a*), 0·15 mm. thick, shows distinct granular structure, and a tendency to form irregular fibres normal to the outer edge. This part gives irregular extinction. The inner portion (2*b*), 0·1 mm. thick, is more uniform in structure, but shows thin layers, which are very thin and distinct near the inner surface. Comparable layers are visible in the adjoining part of the nymphæ.

There is much non-calcareous matter in the shell along the face of the nymphæ to which the internal part of the ligament (2) is joined. Near this junction also the ligament shows a similar well-developed fibrous structure (the " ligamentbandwall," Haas, 1931, p. 237); but the boundary between the ligament and the adjacent shell is nevertheless definite.

STRUCTURE OF *Tellina tenuis* COMPARED WITH THAT OF OTHER BIVALVES.

The shell of *T. tenuis* is unlike that of other modern species of *Tellina* of which the structure is known. *T. baltica* and *T. calcaria* have only two layers, the outer of crossed lamellar structure, the inner of complex type. In these species there is thus no layer corresponding to the outer prismatic layer of *T. tenuis*, a somewhat surprising difference when it is remembered that in this latter the shell is exceptionally thin.

The Tertiary species (*T. ambigua, T. zonaria,* and *T. corneola*) described by Bøggild (1930, p. 288) are more closely comparable with *T. tenuis*, having three layers somewhat similar in character to those described above; in *T. ambigua* and *T. zonaria* the radial prisms of the outer layer are strongly reclined, but in *T. corneola* they show the feathery arrangement seen in *T. tenuis*. In some of these forms there is less distinction between the successive layers than in *T. tenuis*, the prisms of the outer layer being continuous with the lamellæ of the middle layer.

Rather similar structures are thus found throughout the Tellinidæ. Some related families, for example, the Lucinidæ and Donacidæ, have also three comparable layers. On the other hand, there is more variety among the Veneridæ, which have two layers only, the outer layer in some species being

of crossed lamellar structure, in others of radial prisms. Some of the Mactridæ consist wholly of crossed lamellar structures.

The combination of three layers in *T. tenuis* may be related to the extreme thinness of the shell, for they undoubtedly add to its strength. Crossed lamellar structure, as Bøggild has observed (1930, p. 254), affords considerable strength, but a shell consisting simply of a layer of this structure tends to break easily between the lamellæ (along lines following the growth lines). The presence of radial prisms in the outer layer, with their axes across this direction of weakness, and of superposed thin plates in the inner layer, leads to the remarkable toughness of the delicate shell.

The Mantle and Shell Deposition.

Structure of the Mantle.

The term mantle is applied to the tissue which is lined on both sides by epithelial cells. The outer and inner sides are the sides of the mantle which are respectively nearest the shell and the foot.

When the mantle of *T. tenuis* is removed from the shell and mounted flat

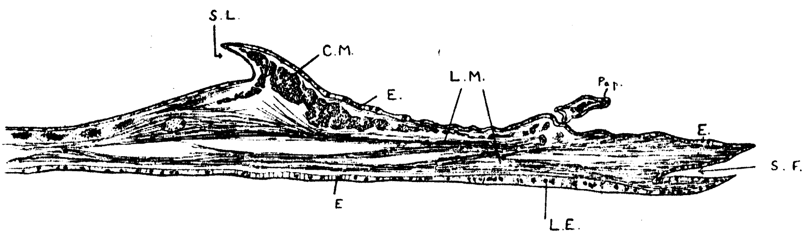

Fig. 9.—The edge of the mantle of *T. tenuis* (×150). E. normal epithelial cells; L.E. large epithelial cells; S.F. small fold at margin of mantle; Pap. papilla or tentacle in section; S.L. small ledge; C.M. concrescent muscle strands; L.M. longitudina muscles. The lower side of the section is outermost in normal position.

it appears to vary from extreme thinness near the umbonal area to a maximum thickness near the margin. This margin may be seen to possess a row of small papillæ about 0·065 mm. by 0·12 mm. Muscle fibres are more concentrated in this thicker marginal band and can be seen to pass in many directions. The only other muscle of importance is the siphonal muscle bands which appear to be fan-like in shape and to radiate from the point of origin, which is the commencement of the siphonal tube on the posterior side of the mantle (fig. 11). In the living specimen, a row of pigment cells may be seen on the inside of the mantle about 1 or 2 mm. from the margin.

The mantle thickness and muscle relationships were more closely examined in transverse section, stained with Delafield's Hæmatoxylin and Eosin. The marginal edge of the mantle is double, its two parts enclosing a small fold (figs. 9, 10) in which the periostracum is secreted. On the outer side of the mantle at a small distance from the tip are a number of enlarged epithelial cells (fig. 9, L.E.) beyond which are normal sized epithelial cells (fig. 9, E.).

On the inner surface the papillæ may be seen approximately opposite these enlarged epithelial cells. Proximally, the mantle thickens considerably to form a small ledge approximately 0·085 mm. deep. Beyond this the mantle is thin. Where no muscle occurs it is about 0·02 mm. thick, consisting of only a double layer of epithelial cells with a small quantity of interstitial tissue (fig. 10).

It was found that the muscles were most easily detected, both in flat

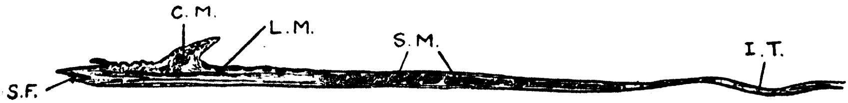

Fig. 10.—Section of part of the mantle of *T. tenuis* (×50). S.M. siphonal muscle bands; I.T. internal tissue. (Other letters as in fig. 9.)

mounts and in sections of the mantle, by the use of polarized light. Muscle fibres are doubly refracting and this affords an easy means of tracing their courses (fig. 11).

For the sake of comparison, measurements have also been made of the mantle of *Mytilus edulis* and *Anodonta cygnea*; a very young specimen of the latter being used (about 50 mm. long). The normal epithelial cells were about

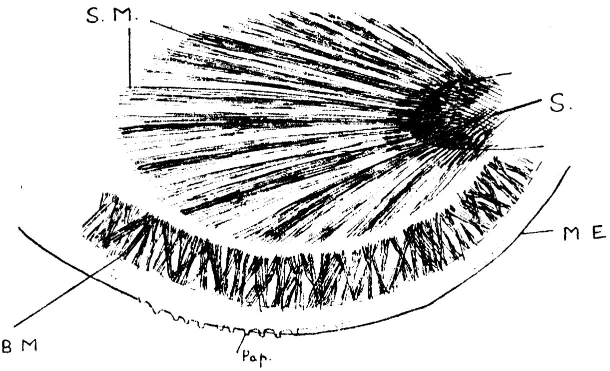

Fig. 11.—Diagram of a flat mount of the mantle showing muscle structures. S. muscular base of siphon; B.M. band of longitudinal muscles; M.E. mantle edge. (Other letters as in fig. 9.)

0·03 mm. deep and the largest were of about 0·05 mm. in depth. Hence, generally speaking, they are considerably larger than in *Tellina*. The average size of epithelial cell near the mantle tip in *M. edulis* was about 0·02 mm., considerably larger than in *T. tenuis*.

Presence of Calcium in the Cells of the Mantle.

By various means attempts were made to determine the distribution of calcium in the mantle. Care was taken to prevent acidity and consequent solution of calcium.

The best results were obtained by the ammonium oxalate method. The procedure was to remove part of the shell so as to allow access of the solution to the mantle. The whole was placed in a solution of 0·5 p.c. ammonium oxalate in water, with salt (NaCl) added up to about the salinity of sea water. Sea water could not itself be used since on addition of the ammonium oxalate a white cloudy precipitate was obtained (probably by the reaction of ammonium oxalate with calcium in the sea water). The bivalve was left in this solution for one hour and then placed in neutral formalin. Both sections and flat mounts were made, and in each calcium oxalate crystals were detected, using polarized light. An attempt was made to combine this procedure with that of Von Kossa ; sections which showed crystals of calcium oxalate in the mantle were placed in silver nitrate solutions, when the silver should replace the calcium. Only very partial success was obtained by this method.

Attempts were also made to precipitate the calcium in the mantle by other means, for example, by the addition of a solution of ammonium carbonate ; no precipitate was observed by this method.

Sections of the mantle of *T. tenuis* prepared by the oxalate method were

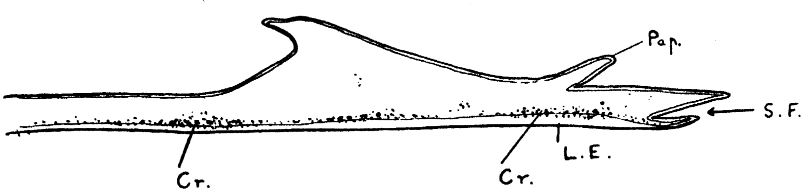

Fig. 12.—Outline drawing of mantle and epithelium with location of calcium oxalate crystals (Cr.) indicated. (Lettering as fig. 9.)

generally stained in Delafield's Hæmatoxylin as a counterstain. With crossed Nicols crystals of calcium oxalate were observed in the mantle, representing the calcium present in the living tissue. The crystals could be seen to extend from the tip of the mantle to the tissue nearer the visceral mass. The crystals were almost all situated immediately below the outer epithelium. In a few examples only were they observed in the epithelial cells or outside the subepithelial region. They appear to be fairly evenly scattered throughout their extent, but they are fewer at the extreme tip of the mantle and then increase for a short distance before being fairly evenly (but less densely) spread over the more proximal part of the mantle. This and the exact relationship of the crystals to the tissue is shown in pl. I, fig. 5, and text-fig. 12.

The size of the crystals of calcium oxalate varies considerably. They vary between 0·002 mm. (or less) and 0·01 mm., being generally about 0·006–8 mm. Their size is probably related to the amount of calcium present at a particular point.

Similar methods of calcium detection have been used by Hayasi (1938) on the mantle of *Anodonta,* and it is interesting to compare his results with those obtained in *T. tenuis*. His procedure was very similar to that described

above, the principal variation being that he used 0·5 p.c. ammonium oxalate and 10 p.c. neutral formalin together in fixing the mantle.

Hayasi found the crystals mainly situated just below the epithelium on the inside of the mantle and scattered to a small extent through the tissues towards the outer surface. Those nearer to the outer surface tend to change in form and decrease in size. Thus he assumed some change of quality takes place in the calcium of the connective tissue at the time of shell deposition. He also observed that since no special gland, except the mucous glands, was seen in the mantle, calcium would be secreted from all parts of the mantle. Crystals, he continues, are not absent from all epithelial cells and vary in proportion through the mantle, which he interprets as meaning that the deposition of calcium may take place rhythmically. There is little evidence for rhythmic deposition in *T. tenuis,* for the crystals are fairly evenly distributed through the mantle.

In *T. tenuis,* moreover, the crystals observed were almost all situated below the epithelial cells of the outer side of the mantle. It was sometimes difficult to be quite certain about their exact position, however, and from Hayasi's photographs this also seems to be the case in *Anodonta.*

In conclusion it must be remembered that these crystals are observed in sections and that some displacement may have taken place in the process of section-cutting.

Method of Deposition of the Bivalve Shell.

Before discussing the bearing of the observations recorded above on theories of shell deposition, it will be convenient to summarize current views and to refer briefly to some recent work on the subject. It is generally accepted that the shell is secreted by the mantle, a conclusion already reached by Reaumur in 1709 and accepted by many workers ; an alternative view, that of Hérissant (1766) and Konigsborn (1877), who maintained that the shell is formed by a direct transformation of tissue, somewhat as in vertebrate bone, now receives no support.

Moynier de Villepoix (1892) recorded the occurrence of two types of cells in the mantle of mussels, first those which secrete calcium carbonate, second those which secrete conchyolin. The conchyolin for the periostracum is normally secreted from a group of cells situated just under the tip of the mantle (see Rawitz, 1892 ; Dakin, 1909 ; White, 1937 ; Manigault, 1939, figs. 34, 35, 39). Secretion of calcium carbonate goes on from the whole of the mantle surface, the inner layer being laid down by the more proximal parts of the mantle and by the epithelium covering the visceral mass.

Recent views on the method of secretion have been summarized by Robertson (1941). Calcium carbonate is extracted from the blood by means of certain cells of the mantle and passes, probably in a colloidal form, through the epithelial cells, to a position between the epithelium and periostracum. Crystallization from this colloidal gel then takes place extracellularly.

Prenant (1924) has recorded that in *Helix pomatia* the cells concerned are leucocytes in the mantle edge ; these produce vaterite spherules which change, when secreted, into calcite.

De Waele (1930) has recently put forward another view concerning the secretion of the shell. He has stated that in *Anodonta cygnea* there is present between mantle and shell a fluid (extrapallial fluid) which constitutes as much as 25 p.c. of the tissues by volume. (This figure seems extremely large when it is remembered that in normal specimens of *Anodonta* the mantle is in fairly close contact with the shell.) The successive layers of the shell, he believes, are derived from the decomposition of a " protéine-carbonato-calcaire " substance, present both in the blood and in the extrapallial fluid. The fluid dissociates by the loss of carbon dioxide on exposure to the air and precipitates conchyolin and calcareous material. He therefore regards shell formation as a similar physico-chemical process. Among other difficulties in this explanation, it must be noted that there is only 4 p.c. organic matter in the shell, but when the extrapallial fluid is exposed to the air the precipitate is 58 p.c. organic material. It is difficult to account for this discrepancy by the resorption of organic matter from the new shell.

Hayasi (1938) has also investigated calcium secretion in the same Bivalve (*Anodonta cygnea*) and has reached conclusions which are strongly opposed to the views of De Waele : unfortunately he does not seem to have been aware of the latter's work, but his results appear to be in agreement with the more orthodox theory of calcium secretion.

P. Manigault (1939), in an extensive histological and chemical study, has supported the views of De Waele so far as to emphasize the importance of the extrapallial fluid. He suggests that this fluid, in contact with a " protéique calcaffine " matrix, decomposes and gives up its calcium carbonate. He believes that the fluid results from the activity of an enzyme which he compares with the phosphatase concerned in the ossification of bone. As Manigault noted, however, Bivalve shells contain a very small proportion of calcium phosphate (Turek, 1933, p. 298, recorded only 0·00049 p.c. of phosphorus in the shell of *Anodonta*), while in bone 90 p.c. of the dry inorganic matter is calcium phosphate (Halliburton, 1939).

The view that deposition results from secretion from the part of the mantle immediately adjacent and not simply by precipitation from an extrapallial fluid appears to be borne out by the thickness changes observed in the outer and middle layers during growth. For if deposition were from an extrapallial fluid it is conceivable that crystals would continue to extend according to the characteristic types of these layers, but it is unlikely that their thicknesses would be increased proportionately after each growth halt. It seems much more probable that the thickness changes are the direct result of increases in the areas of the mantle concerned with their secretion.

The writer therefore believes that the most probable method of formation of the shell is by the secretion of a calcium salt from the subepithelial tissue in colloidal form. This colloid may be transformed into shell material either

by enzyme action, or by a physico-chemical reaction, or by a combination of the two.

From the observations recorded above, it is clear that in *T. tenuis* the calcium carbonate is secreted through the epithelial cells of the outer surface of the mantle from the subepithelial tissues. There appear to be no special cells present near the mantle edge for secretion. The eosinophile cells described in the mantle of *Pecten* (Dakin, 1909, and Manigault, 1939) are not present. The epithelial cells themselves show no special modifications either for the secretion of the periostracum, under the tip of the mantle, or the secretion of calcium carbonate, except for enlarged epithelial cells (p. 82 and fig. 9, L.E.) and a general tendency for the cells of the outer (secreting) surface of the mantle to be slightly larger than those of the inside.

In any theory of shell formation it is important that attention should be paid to the nature of the various layers of the shell and their structures. For example, in *T. tenuis*, there are, in addition to the periostracum, three layers of different character. It must be supposed that the outer layer is laid down from the epithelial cells of the region of the edge of the mantle, that the middle layer is formed (either simultaneously or almost simultaneously) by more proximal cells, and that the inner layer is laid down from the other epithelial cells of the mantle and from the epithelial cells covering the visceral mass. In relation to these features, it is interesting to observe that there is no variation in the size and shape of the epithelial cells, except for a group of larger cells near the edge of the mantle.

Various theories have been put forward to attempt to explain the occurrence of the different shell layers. They may be summarized :

1. By variation of the nature of the epithelial cells.
2. By the structure of the base on which deposition occurs.
3. By concentration of salts on deposition.

The first idea has already been partially dealt with. There is little sign of variation of cell structure in different parts of the mantle. Boutan (1923), in his experiments on regeneration, has shown that on regeneration, after removal of the whole shell, first the periostracum, then the layers of calcareous material in normal succession, are laid down over the whole surface of the mantle. This means that any area of the mantle may secrete any type of structure when this is necessary. It seems that some factor independent of the precise part of the mantle causes the deposition of the shell layers of the various types.

A somewhat similar theory to the second of those given above has been recently advanced by Manigault (1939, p. 385). He says that there exists between the mineral of the regeneration and the organic part which precedes and accompanies it, a co-operation which is entirely analogous to the relationship of bone and its protein-like matrix before ossification. Although no sign of structure was observed in the periostracum of *T. tenuis*, it must be remembered that it is very delicate and extremely thin.

The third theory is of great importance. It may be considered in two

main ways. The first view is that of De Waele (1930), who suggested that the extrapallial fluid was diluted near the shell border by the water of the environment; he held that this passes through the free border of the periostracum by osmosis into the " space " between the mantle and shell, thus diluting the extrapallial fluid to different extents according to its proximity to the edge of the shell. Thus from extrapallial fluid of varying concentrations the various layers are regularly laid down. In this connection it is difficult to see how the successive shell layers are laid down when undergoing regeneration. According to De Waele's view also the pallial line should mark a change of concentration and the inner layer should only occur within it. In *T. tenuis* this is definitely not so, for the inner layer is secreted both inside and

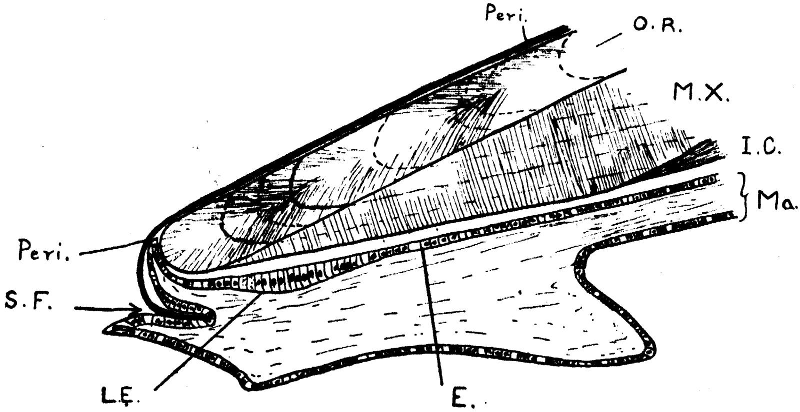

Fig. 13.—Diagram showing approximate relations of shell and mantle (Ma.); also shows the origin of the periostracum in the small fold at the margin of the mantle. (Lettering as figs. 2 and 9.)

outside the pallial line; in some shells, however, this does not appear to be the case (see Rubbel, 1912, fig. C).

A rather different interpretation of the idea that varying concentrations cause layers of different structure is possible on the supposition that the calcium salt secreted by the cells is in colloidal form. The concentration of this colloid may vary in different regions and at different times (i.e. during regeneration) and so produce various crystal forms. It is worth while pointing out that the main axis of the crystals normally tends to be perpendicular to the secreting surface of the mantle. This may not be apparent in the outer layer of the shell of *T. tenuis* but the mantle would bend round the curved edge (fig. 18).

In this connection recent conclusions by Bryan and Hill regarding skeleton formation in the Hexacorals are of some interest: they consider that the skeleton is " due to the organic guidance of an inorganic process," the number and position of the trabeculæ being decided by the distribution of the active centres of calcification (1941, p. 90). Their conclusion that the

relation of the fibres of the skeleton " to the colloidal matrix secreted externally by the ectoderm has its parallel in the relation of the fibres of a spherulite to its mother liquor " (p. 89), is of interest in connection with the somewhat spherulitic structure of the outer layer in *T. tenuis*.

The Deposition of Aragonite.

A further problem in shell secretion is raised by the occurrence of different forms of calcium carbonate, calcite, and aragonite. At present no explanation can be given for this difference. It has been suggested that it may be related to differences of salinity, temperature, and to the presence of additional substances.

The possible importance of difference of salinity is indicated by the fact that few, if any, Bivalves living in fresh water have calcite present in the shell. Moreover, the only fresh-water genera of the Mytilidæ (*Dreissensia* and *Congeria*) are composed of aragonite, though calcite occurs in the marine forms of this family. It may be noted, however, that when *M. edulis* is bred in water which is of the minimum salinity, it develops a thin shell containing both calcite and aragonite in the proportions normal to the species (Bøggild, 1930, p. 242). Moreover, since species with shells of calcite co-exist in seas with species having aragonite shells, it is unlikely that salinity is a major factor in determining the nature of the shell.

Further, temperature may be an essential factor in the formation of inorganic calcite and aragonite, but seems to be of little consequence in its organic deposition, for both calcite and aragonite are formed in shells of organisms living in a wide range of temperature. There is a tendency, however, for the formation of calcite at lower temperatures than aragonite (Kendall, 1896, p. 790), and it is possibly significant that *Ostrea* sp., which has a calcite shell, only grows between the temperatures of 10–15° C., and at higher temperatures stops growing (Orton, 1928). Further observations on other species may throw more light on this problem.

A more probable factor in the production of aragonite and calcite may be the influence of other substances. Prenant (1927) mentioned the possibility of calcium sulphate influencing the form of the calcium carbonate. More important may be the fact that magnesium is found, in varying amounts, in many calcite shells but never in shells of aragonite (Clarke and Wheeler, 1917; 1922): magnesium and calcium carbonates are isomorphous, and magnesium carbonate may be present in solid solution in calcite. Although some calcite shells have practically no magnesium, it may be emphasized that the presence of magnesium carbonate in the salts secreted may determine its crystallization as calcite and not as aragonite (see also Manigault, 1939, pp. 380, 419).

The possibility that other substances may similarly determine the formation of aragonite led to the spectroscopic examination of powdered shells of various types. Particular attention was paid to the possible occurrence of strontium, of which traces were observed in the following shells :—

(*a*) *T. tenuis* (rather small traces, but recognized definitely).

(*b*) *T.* (*Macrotoma*) *baltica* (more definite than (*a*)).

(*c*) *Donax vittatus.*

The calcite shell of a specimen of *Ostrea edulis* was also tested but no strontium was observed.

A greater range of spectroscopic tests is needed to establish the distribution of strontium in bivalves, but it is suggested that its presence may contribute towards the formation of aragonite. It may be noted that strontium carbonate, like calcium carbonate, forms orthorhombic crystals, and that mineral aragonite frequently contains a small percentage of strontium carbonate. Moreover, it has been stated that aragonite will form from solution at normal temperatures if small amounts of strontium or lead carbonates are present (Dana, 1932, p. 522).

It is believed that the occurrence of small proportions of magnesium or strontium carbonates, or perhaps of other salts, may influence the formation of calcite or aragonite in shells.

Conclusions.

1. The shell of *T. tenuis* (in addition to a thin periostracum) consists wholly of aragonite. This is in three layers, viz. :—

Outer layer of composite prismatic structure.

Middle layer of crossed lamellar structure.

Inner layer of complex and homogeneous structure.

Only two layers are present in thicker modern species of this genus, though three similar layers are found in other thin shells of the Donacidæ and Lucinidæ. It is suggested that the structural complexity is related to the strengthening of the delicate shell.

2. Thickness changes in the layers in relation to growth stages are recorded. It is shown that the outer and middle layers become thicker towards the shell margin, while the inner layer becomes thinner in the same direction.

3. The microscopic characters of the horny ligament are described. It shows strong double refraction and (in the inner part or resilium) pleochroism.

4. The mantle is extremely thin, 0·02 mm. away from the edge and where no muscle fibres are present. Near the margin the mantle is thickened and in one region the epithelial cells are larger. The average epithelial cells are smaller than in *Mytilus edulis* and *Anodonta cygnea.*

The presence of calcium salts in the mantle was shown by reaction with ammonium oxalate solution, followed by examination with polarized light. Most of the calcium appears to have been situated immediately below the outer epithelium.

5. The various crystal elements in the shell layers were deposited approximately at right angles to the face of the mantle secreting them. The " extrapallial fluid " theory of De Waele does not seem to account for the different

structures ; neither does the influence of the structure of the periostracum (as suggested by Manigault) appear adequate to explain the structure of the layers, except possibly the outermost layer.

6. The presence of traces of strontium in this and some other aragonite shells is recorded ; the known occurrence of magnesium in many calcite shells suggests that non-calcareous substances may influence the formation of calcite or aragonite. Temperature and salinity may also be significant in this connection.

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DESCRIPTION OF PLATE I.

Fig. 1.—Horizontal section of outer layer (radial prismatic structure) of *T. tenuis*. With crossed Nicols. × 200.

Fig. 2.—Horizontal section of middle layer (crossed lamellar structure) of *T. tenuis*. With crossed Nicols. × 150.

Fig. 3.—Radial section of shell of *T. tenuis*, showing the shell margin with periostracum and the three shell layers. The section is rather thick and the opaque bands appear dark. × 15.

Fig. 4.—Section of the ligament of *T. tenuis*. ×80. The internal part of the ligament is torn away from the shell. (*Cf.* text-fig. 7.)

Fig. 5.—Section of the mantle of *T. tenuis*, after treatment with ammonium oxalate solution. The photograph was made by using first transmitted light, then crossed Nicols, crystals of calcium oxalate thus appearing white (only the larger crystals are visible). × 300.

VI.—A REVIEW OF CRYSTALLOGRAPHIC MICROSCOPY. 548:535

By EDWIN E. JELLEY, D.Sc., F.I.C., F.R.M.S.

THE present review is intended to give a general summary of the use of the polarizing microscope and its accessory apparatus in various applications of optical crystallography, with particular reference to chemical microscopy.

Petrography has been an important factor in the development of the polarizing microscope. Ever since 1857 when, according to Hadfield (1920), H. C. Sorby described his microscopical studies of rock sections to an unappreciative audience before the Geological Society of London, a great deal of attention has been devoted to working out qualitative and quantitative methods of identifying the mineral constituents of rocks. The polarizing microscope, at first little more than an ordinary microscope fitted with a polarizer and analyser, rapidly developed after 1880, when precision methods of crystallography began to be applied to the microscopical examination of small crystals. It will be convenient in this review to consider some of the main developments in various components of the polarizing microscope, without attempting to compile an exhaustive bibliography on the history of the development of the instrument as a whole.

The discovery by W. Nicol (1828, 1839) that a rhomb of calcite could be made to yield a single image, marked a great step forward in the production of plane polarized light. Research was soon directed towards the improvement of the Nicol prism, in order to eliminate so far as possible two main drawbacks: the great length of the prism relative to its aperture, and its steeply inclined end faces which, in the case of the analyser, cause lateral displacement of the microscopical image. Of the many different prisms which were devised, two have been selected in the course of years. The Glan-Thompson prism, which has features due to Glan (1880) and Thompson (1881, 1886), has end faces at right angles to the angle of vision, and in which the optic axis of the calcite is parallel to the end faces and in the plane of the canada balsam or oil film separating the two faces of calcite. The Ahrens prism (1886) resembles the Glan-Thompson in general principle, but has two section planes, whereby the same aperture is attained with a prism of half the length. The dividing line through the centre of one end face of the Ahrens prism is a disadvantage when the prism is used as an analyser, as it is often visible in the field of view. In Ahrens' original construction, the optic axis of the calcite was at right angles to the dividing line, but in all the Ahrens prisms examined by the author in recent years, the optic axis and dividing

line are at 45°, which cause needle crystals having parallel extinction to give diffraction images in the diagonal position.

When a Nicol, Glan-Thompson or Ahrens prism is interposed above the objective in a focused microscope system, refocusing becomes necessary and the magnification of the system is changed. As it is the extraordinary ray which is transmitted by the prism, its equivalent optical path length is different for axial rays in the plane of the optic axis of the prism and axial rays in a plane at right angles to it. This means that axial rays in the plane of the optic axis come to a focus at a greater distance from the objective than do axial rays in other planes. The result shows itself as an astigmatism of the image which is particularly troublesome with medium- and high-powered eyepieces. The astigmatism is greater on one side of the field than on the other with the Nicol prism. It is symmetrical with the Glan-Thompson and Ahrens prisms, and may be approximately corrected by means of a cylindrical lens. However, the best method of overcoming astigmatism is that described by Becher (1915), who placed a negative lens above the objective in order to render the rays of light parallel in their passage through the analyser prism. The rays were then focused by a positive lens to yield an image in the correct position relative to the eyepiece. By having the negative and positive lenses permanently mounted in the body tube of the microscope, an analyser prism may be inserted between them without affecting focus, magnification or location of the image. An analysing Nicol may be used above the eyepiece without introducing astigmatism, if the microscope and eye are focused for an image distance of infinity. However, this position of the analyser is inconvenient with high-powered eyepieces.

Strongly dichroic crystals may be used as polarizers provided that light vibrating in one direction suffers little or no absorption. Certain types of tourmaline have this property. Biot (1815) first suggested the use of dichroic tourmaline as a polarizer, thereby originating the " tourmaline tongs," so important to early workers. Herapath (1852) discovered that thin crystals of quinine sulphate periodide (" herapathite ") were almost colourless for one vibration direction and almost completely absorbing for the other. He proposed to construct polarizers from large thin crystals, but the problem of producing large areas of uniform polarizing power remained unsolved until recently, when Land patented methods of orienting microscopically-small crystals of herapathite and other periodides in films of a plastic such as cellulose acetate. Descriptions of the Land and other polarizing films have been given by Levey (1939). The Land polarizing material has now reached a high state of perfection and, when mounted between optical flats in a medium of the correct refractive index, makes a very good analyser. For use as a polarizer it is not necessary to mount it between optical flats, as a good grade of sheet glass will serve for this purpose. The earliest type of Land polarizer transmitted a considerable amount of unpolarized red light—a characteristic of the dichroism of herapathite—but this defect has now been overcome and only a slight amount of the extreme ends of the spectrum is transmitted by

crossed polarizer and analyser constructed of the Land "Polaroid." Marks and Bernauer have produced polarizing plates from oriented large crystals of herapathite and its cinchonine and cinchonidine analogues. However, the present Land material far surpasses any of these other types of polarizing plates. So far as the author has been able to discover, the slight residuum of unpolarized light transmitted by the latest Land "Polaroid" does not in any way affect the performance of the polarizing microscope, as all of the phenomena in both parallel and convergent light are clearly observable, and the interference colours are not changed. Undoubtedly, the future will see considerable simplification in polarizing microscopes for both petrographical and chemical purposes. For instance, in place of the Becher negative-positive lens system for removing astigmatism, it is only necessary to have a Land polarizer interchanging with a glass plate of the same refractive index and total thickness, as a body tube analyser. For a polarizer it is possible to use a relatively large area of film, so that simplified condenser systems may be used.

Synchronous rotation of polarizer and analyser was first introduced by Dick (1888), to whom also must be credited many other improvements in optical and mechanical construction of the petrographic microscope which still bears his name. Synchronous rotation was also used by Leiss (1895) and Wright (1910). Although the original purpose of synchronous rotation of the nicols was to render unnecessary the rotating stage and the need for centration of objectives and condensers, it is now customary to provide both the synchronous rotation of polarizer and analyser and a rotating stage on microscopes for advanced petrographic work, in order to facilitate universal stage technique.

Substage condensers for use in conjunction with a polarizing prism are now usually constructed so that the top lenses may be swung out of the illuminating beam. This device, due to Wülfing (1889), permits the rapid transition from low-power orthoscopic to high-power conoscopic observation of crystallographic objects. From the point of view of illumination, this system has the disadvantage that an auxiliary condenser is required when the high-power condenser is used, so that the transition is not as convenient as it would be if two entirely separate par-focal condensers were made to interchange by means of a lever or the like.

Another important feature of the polarizing microscope is the Bertrand lens (1878) which, for advanced work, is provided with an iris diaphragm and centering screws. It should be placed as high as possible in order that the conoscopic figures of small crystals may be studied. Only a few of the highest priced petrographic stands have a Bertrand lens suitable for the study of small crystals, such as are usual in chemical microscopy.

The measurement of birefringence is made with the aid of a compensator, the principle being to introduce into the field of view a known degree of birefringence which restores the extinction of the crystal under examination. The earliest type of compensator was the quartz wedge of Biot (1814). In

order to overcome the difficulty of grinding a quartz wedge thin enough to get birefringence of less than a quarter wave length, Wright (1902) combined a quartz wedge with a selenite plate of uniform thickness so oriented that the fast vibration directions were at right angles. The Wright combination wedge has zero birefringence in the centre with increasing birefringence on either side—one side having its fast vibration parallel and the other at right angles to the length of the wedge. This wedge, which was graduated to give direct readings of retardation in μ, is best used in conjunction with the Wright ocular (1910), in which it slides in the focal plane of the ocular. The Johannsen quartz-mica wedge (1910) is based on a similar principle but has zero birefringence at one end. The Babinet compensator, described by Jamin (1850), is a special ocular containing a fixed and a movable quartz wedge having their fast vibration directions at right angles. For the measurement of small degrees of birefringence, compensators constructed on two different principles are now available. The Berek (1913, 1924) compensator, an improvement on the earlier one of Nikitin (1910), consists essentially of a thin plate of calcite cut at the normal to the optic axis, which is rotated about a diameter by means of a graduated drum. This compensator is inserted in a slot above the microscope objective and so does not entail the use of an analyser over the ocular as is the case with most of the compensators previously mentioned. An elliptical compensator is described in Rinne-Berek's book (1934). It derives its name from its application to the study of elliptical polarization met with in the study of metals and ores. It consists of a mica disc having a retardation of 1/30 to 1/10 wave-length, which rotates about the microscope axis.

The measurement of optic axial angles and determination of the optic character of uniaxial and biaxial crystals are amongst the most important of petrographic determinations. A great deal can be done by studying the interference figures given by crystals in convergent polarized light by using a Bertrand lens to observe the back lens of the microscope objective. Fairly accurate measurements of these interference figures can be made using the apparatus and technique described by Wright (1911). Much more precise observations can be made by means of apparatus capable of rotating the crystal about various axes. Leeson (1848) described the first rotation apparatus, which was used to rotate crystals into the proper positions for measuring interfacial angles. Highley (1856) described an inverted chemical microscope in which the rotating stage was a part of the microscope construction, an idea which was later adopted by several instrument makers. With these earlier forms of rotation apparatus, the refraction of light from the crystal into air must have been a source of trouble. One way of overcoming this trouble is to immerse the crystal in a liquid of approximately the same refractive index. Nägeli and Schwendener (1867) first suggested having the rotation apparatus in a trough which could contain water or some other liquid. Amongst the many other workers who contributed to the development of rotation apparatus, two names are particularly prominent. Klein

(1891) devised a number of different types of rotating stages, amongst which may be mentioned a simplified one-axis apparatus for rotating small crystals immersed in liquid, a more elaborate two-axis apparatus for the examination of gems, and a goniometric head for measuring crystallographic angles. Von Federov (1891 to 1898) devised the universal stage as we now know it. Originally, this had three axes of rotation. The crystals, mounted on microscope slides, were held between two spherical segments of such shape that, when combined with the stage plate and microscope slide, their surface was centered with respect to the axes of rotation. The idea of using spherical segments was not new, Adams (1875) having used them in an apparatus for determining optic axial angles of large crystals. Further improvements to the Federov stage have been effected by Wright (1907), Johannsen (1918), and Emmons. The universal stage, as modified by Emmons (1929, 2) and described in the textbook by Winchell and Emmons (1931), simplifies certain optical crystallographic determinations by virtue of its having five axes of rotation, or six, if we include the rotation of the microscope stage. In addition to the Klein goniometer mentioned above, the Dick petrographic microscope has been equipped with a goniometer and other improvements by Miers, Bowman, Grabham (1910), and Tutton (" Crystallography," Tutton, 1922).

The petrographic microscope has been used to estimate the proportions of different mineral constituents by measuring their areas. The principle followed is that of Delesse (1847), which is based on the assumption that the total area of each of the constituents in a section of a rock of uniform composition is proportional to their actual volumes. However, his method was a macroscopic one. The first microscopic method was by means of Hirschwald's planimeter ocular (1904). Typical of modern apparatus is Shand's integrating stage (1916) which has a number of micrometers (usually six) operating one direction of travel of the stage plate ; each of these micrometers is used for some particular constituent of the mineral specimen.

An important aspect of crystallographic microscopy is the determination of the principal refractive indices of crystals. Microscopic immersion methods are used for determining the refractive indices of small crystals which could not be measured on the Abbé crystal refractometer. These immersion methods date from the work of Maschke (1872, 1880), who described the appearance of crystals immersed in liquids having refractive indices lower, higher, or the same as the crystals'. Becke (1893) first described the appearance of fringes of light seen around immersed crystals when the microscope objective was raised or lowered, an effect now known as the Becke line. Schroeder van der Kolk (1898) did much to popularize the immersion method of determining refractive indices. Since that time, a large number of workers, including Wright (1911), have made numerous modifications of the method. The usual method of determining refractive indices was to use a set of liquids covering all values between about 1·45 and 1·75 in steps of 0·005. This system had the disadvantage that it was necessary to remove all of one

immersion liquid from crystal fragments before applying the next, so that recent work has been mainly directed towards reducing to a minimum the amount of manipulation required. Jelley (1934, 2) made use of liquids of very high boiling-point having widely spaced refractive indices. These were mixed in a cavity on the microscope slide containing the crystal fragments, until the Becke line effect showed that the refractive index for a particular vibration plane had been matched. A droplet of the liquid mixture was then transferred to a micro-refractometer and the refractive index read with an accuracy of one or two units in the third decimal place. The liquids specified covered the range of 1·410–1·742. The micro-refractometer consists of a bevelled cover glass in contact with a glass slide and so arranged that the small drop of liquid forms a compound prism with the 45° bevel on the glass. This micro prism is placed over an aperture somewhat wider than the prism. When one looks through this aperture towards an illuminated slit, a virtual image of the slit is seen superimposed on a scale, so that the refractive index can be read directly. Kunz and Spulnik (1936) also proposed the use of liquids of very high boiling-point and approximately similar volatility for making refractive index mixtures. In addition to the liquids mentioned by Jelley, they suggest the use of mixtures of heptylic acid and α-bromo-naphthalene to cover the range 1·423–1·658.

More accurate methods of matching the refractive index of crystals by means of immersion liquids are possible by what are known as the "index variation" methods. The single-variation method of changing the wave-length of the microscopic illumination by means of a monochromator was proposed by Posnjak and Merwin (1922) and subsequently developed by Tsuboi (1923). In this method an immersion liquid is found which is near enough to the correct refractive index to give a colour fringe with the Becke line in white light. A monochromator is then used to determine the exact wave-length at which the refractive index of the liquid matches that of the crystal. Emmons (1928; 1929, 1) introduced a single-variation method in which the temperature of the immersion liquid is raised until its refractive index falls to that of one of the indices of the crystal fragments, and a double-variation method in which the index is roughly adjusted by temperature variation after which an accurate adjustment is made by varying the wave-length. This double-variation method has the particular advantage that a greater refractive index range is available without changing the immersion liquid. In both of these methods, the actual refractive index of some of the immersion liquid was measured on an Abbe refractometer, the prisms of which are kept at the same temperature as the cell containing the immersed crystal fragments. Emmons (1929, 2) described a modified universal stage for use with the double-variation method, and also (1929, 3) a set of thirty immersion media specially chosen for their high dispersion and high temperature coefficient of refractive index.

Optical crystallography is an important aspect of chemical microscopy. In some branches of microchemistry an ordinary microscope is used as an aid

in performing very delicate operations on minute quantities of materials. Such, for example, is the principal technique of Benedetti-Pichler, whose work, however, falls outside the scope of the present review. Chemical microscopy, as taught in Cornell University, includes an adequate training in the more elementary aspects of crystal optics, and the students are made familiar with the polarizing microscope. These courses in chemical microscopy have been built up by Chamot and Mason, whose " Handbook of Chemical Microscopy " must be ranked as one of the big achievements in the application of the polarizing microscope to problems in analytical chemistry. Hartshorne and Stuart (1934) have also published a book on the study of crystals by the polarizing microscope from the point of view of the chemist. In this book the emphasis is on the optical, rather than the chemical, aspects of the subject. On the purely crystallographic side, is the work by Winchell and Emmons previously referred to.

Until recently, chemical microscopy mainly consisted in applying optical methods to the observation of chemical reactions of the material under test, and was largely limited to the study of inorganic substances. In organic chemistry, the study of the optical properties of crystals has relatively greater importance as few organic reactions can be carried out under the microscope, whereas the optical properties of organic crystals show a much greater variation of properties with change of wave-length than is commonly met with in inorganic crystals. It is natural, therefore, that attention is now being given to studying the optical properties of crystals of organic compounds with various wave-lengths of light. Bryant (1932, 1933, 1935, 1936, 1938, 1939) has described the optical properties of a number of organic compounds in which particular attention was paid to the effect of varying wave-length. He has described a photographic method (1941) of measuring different types of selective dispersion in organic compounds. Bryant and Mitchell (1938) measured the optical crystallographic properties of a number of *p*-bromoanilides. Jelley (1934, 1; 1936, 1; 1941) described three different forms of a grating microspectograph specially designed for recording the optical properties of crystals for the entire visible spectrum. In the first paper, a method of determining optical axial angles as a function of wave-length, and a method of determining the birefringence of crystals throughout the spectrum as a means of identification, were given. In the second paper, a more elaborate form of the instrument was described which used a transmission replica grating. The spectra were photographed on 35-mm. film in order to get considerably shorter exposures. This instrument was equipped with a Wollaston prism in order to obtain direct photographic records of dichroism. In the third microspectrograph a speculum refraction grating was used and a modified optical system employed in order to get greater accuracy in wave-length measurements. The microspectrograph has been applied to the study of absorption phenomena in crystals and polarization phenomena both in parallel and convergent light. The application of the microspectrograph to the study of pleochroism of crystals of rare earth salts

was given by Jelley (1935), who also described its application to the study of a mesomorphic phase of a cyanine dye (1936, 2; 1937).

In conclusion, mention must be made of the growing interest in the determination of optical constants of absorbing crystals by means of polarized vertical illumination. Königsberger (1909) was the first to apply polarized light to the microscopic study of metals, and Wright (1919) described the technique of ore and metal examination by this means. An elementary account of the theory and use of polarized reflected light was given by Dayton (1935), whose paper gives references to other workers' publications. The most comprehensive treatment of the theory and measurement of such optical properties as may be determined by polarized vertical illumination, has been given by Berek (1937).

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VII.—CULTURE OF WHOLE ORGANS. 591. 3. 4.

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(*Centenary Communication, Royal Microscopical Society*, 1839–1939).

TWO PLATES AND FOUR TEXT-FIGURES.

BIOLOGICAL progress has been achieved, during the last century, along two different lines of research : the study of structure and the study of function. These two lines have tended to merge during the last two decades. The morphologist is no longer occupied by merely describing what he observes in this two-dimensional world of his, the microscopical section. He is not even content by adding to his technique a third dimension which is tantamount to serial sections and reconstructions. He has become histophysiologist, and as such he must add Time to his observations.

There are many ways to do this. Given a suitable apparatus it is possible, for example, to bring the microscope to bear upon a number of different organs *in vivo* and *in situ*, and it is feasible therefore to follow visually, during a short time, some of the biological phenomena connected with the specific action of the organ concerned. For many reasons—technical as well as scientific—it is desirable, however, to apply an experimental technique which will enable such work to be carried out on *isolated* organs or tissues.

The method of arterial perfusion of excised organs did not originate in the histological laboratories. It is well known that the German physiologist Ludwig and his pupils were the pioneers in this field. Ludwig designed brilliant experiments by which the function of isolated organs could be studied. Subsequent physiologists have modified and improved the technique of perfusion and artificial circulation, the crowning achievement being, of course, the Starling heart-lung preparation. But all these methods were designed exclusively for use in the physiological laboratory. Histological examination of the tissue to be studied was of no consequence. The experiments are essentially of the short duration type ; the survival of the organs is limited mostly to a few hours. There is no safeguarding against infection. They are definitely not designed for *culture* of tissues or organs.

What the histophysiologist needs is, however, experiments of longer duration—observation periods extended to days or weeks instead of hours—which will enable him to study growth and development, regeneration, dedifferentiation, or degeneration ; endocrinology ; inflammatory reactions, etc. Such culture methods for whole organs have recently grown out of the established techniques for tissue culture.

It is common knowledge how Ross Harrison inaugurated a new era in histology by the introduction of his method for explantation of living tissues *in vitro*. At the time of Harrison's epoch-making experiments on amphibian nerves, Carrel had nearly completed his important research on vascular surgery and made the first successful transplantations of whole organs (e.g. kidneys or whole extremities). At that period biologists had clearly realized the importance of the phenomenon which Osterhout aptly has termed the "little deaths." A complex biological entity does not die at once. The heart will fail; then the nervous system will commence to disintegrate; but long after the highly differentiated organs have ceased to live, connective tissue, bone, etc., will retain a certain degree of vitality. Thus the death of an entity is really a succession of "little deaths." By securing samples of living tissues for explantation their survival is rendered possible for long periods after the death of the individual.

Why not try to keep whole organs alive outside the body? This is exactly what Legallois * thought when he wrote the prophetic lines in 1812 about the possibility of artificial circulation. Carrel † had attempted, during his work on transplantation, to keep isolated organs *in vitro* under nearly natural conditions. For various reasons these experiments were discontinued, and instead he and his pupils brought the explantation method up to a very high standard. Looking back, it may be said to-day that tissue culture enabled cytologists to keep mammalian cells alive *ad infinitum*. But it became evident that "tissue" culture meant really *cell* culture. Many modifications were suggested in order to ameliorate the culture of organized tissues, the de Haan circulation apparatus and the Lewis roller tube method being examples of such efforts of experimenting cytologists in recent years.

In spite of these improvements many biologists realized that if *in vitro* experiments were to be of any use for histophysiological research they would have to be carried out with organized material of which the elements retain their histotopography. To obtain this, means for abundant nutrition and oxygenation must be provided. It became clear, therefore, when culture of organs was contemplated, that a special oxygenator pump would have to be constructed. The pulse should be rendered faithfully like the natural circulation with correct systolic and diastolic amplitudes. The oxygenation should be as complete as possible. And, above all, the whole apparatus must be germ-proof.

Experience in Carrel's laboratory had shown that an artificial circulation over prolonged periods cannot be kept up under aseptic conditions by the old-fashioned arrangements with pumps and pistons. The vascular bed of the excised organ must be fed by a culture medium circulating in a closed system entirely separated from the power section of the apparatus. After a great deal of preliminary research Charles A. Lindbergh found that the use of

* Legallois, C. J. J., "Expériences sur le Principe de la Vie," D'Hautel, Paris, 1812 (quoted from Carrel).

† Carrel, A., "Concerning Visceral Organisms," *J. Exp. Med.*, 1913, **58**, 155.

compressed air, acting through the intermediary of a siphon oil-valve—is the best way to convey systole-diastolic oscillations to the arterial system under

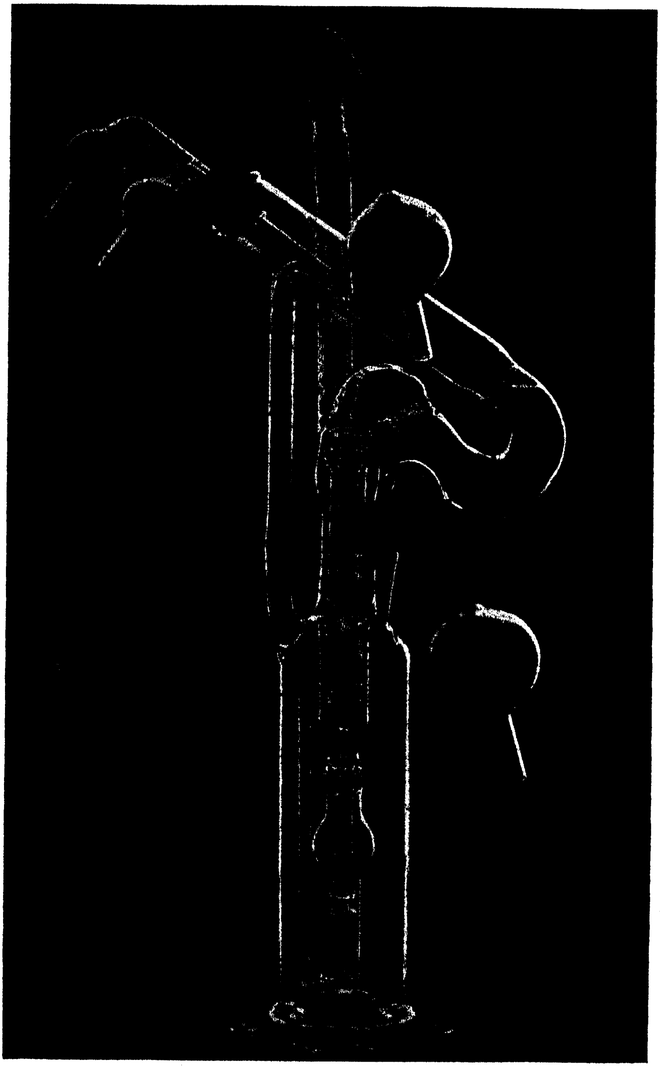

Fig. 1.—Showing the Lindbergh pump assembly.

aseptic conditions. This ingenious apparatus (fig. 1) was perfected in 1935–36 and is known as the Lindbergh pump (fig. 2). It is not very difficult to operate. But to utilize it so that dependable results are obtained requires a

highly-trained technical staff. Simplified modifications of the Lindbergh pump have since been designed; by Nicholas at Yale; and by Thomas* at the Fondation Curie (fig. 3). The Lindbergh method for culture of whole organs is the only one with which I have had personal experience. Therefore, I wish to limit my description to this apparatus. It is of what I saw as a histologist, working with Carrel's group at the Rockefeller Institute for Medical Research, that I propose to write.

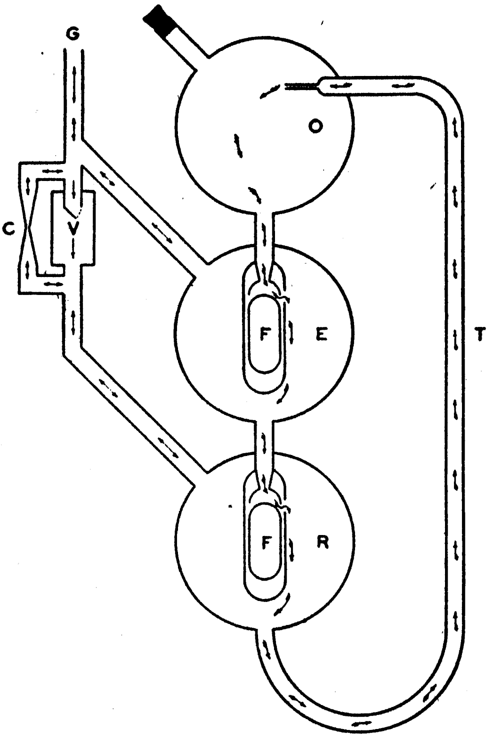

Fig. 2.—Diagram of the Lindbergh pump. O, organ chamber. E, equalization chamber. R, reservoir chamber. F, floating valves. T, ascending feed tube. G, gas line. C, constricted opening. V, one-way valve.

It may be appropriate first to describe very briefly the manner in which the oxygenator pump is operated. For details one should consult the original papers.†, ‡, §, ||, ¶

* Thomas, J. André, "Un appareil pour la perfusion stérile et automatique des organes," *Annales de Physiol.*, 1938, **14**, 799.

† Lindbergh, Charles A., "Apparatus to circulate liquid under constant pressure in a closed system," *Science*, 1931, **73**, 566.

‡ Carrel, A., and Lindbergh, C. A., "The Culture of Whole Organs," *Science*, 1935, **81**, 621.

§ Lindbergh, C. A., "An Apparatus for the Culture of Whole Organs," *J. Exp. Med.*, 1935, **62**, 409.

|| Carrel, A., "Technique of the Culture of the Thyroid Gland," *J. Exp. Med.*, 1937, **65**, 515.

¶ Carrel, A., and Lindbergh, C. A., "The Culture of Organs," Hoeber, New York, 1938.

Inside the superior chamber, into which is tied the organ to be cultivated, such glands or anatomical parts may be placed, of which an arterial stem can be laid free by dissection. It will be noticed that only the arterial side of the

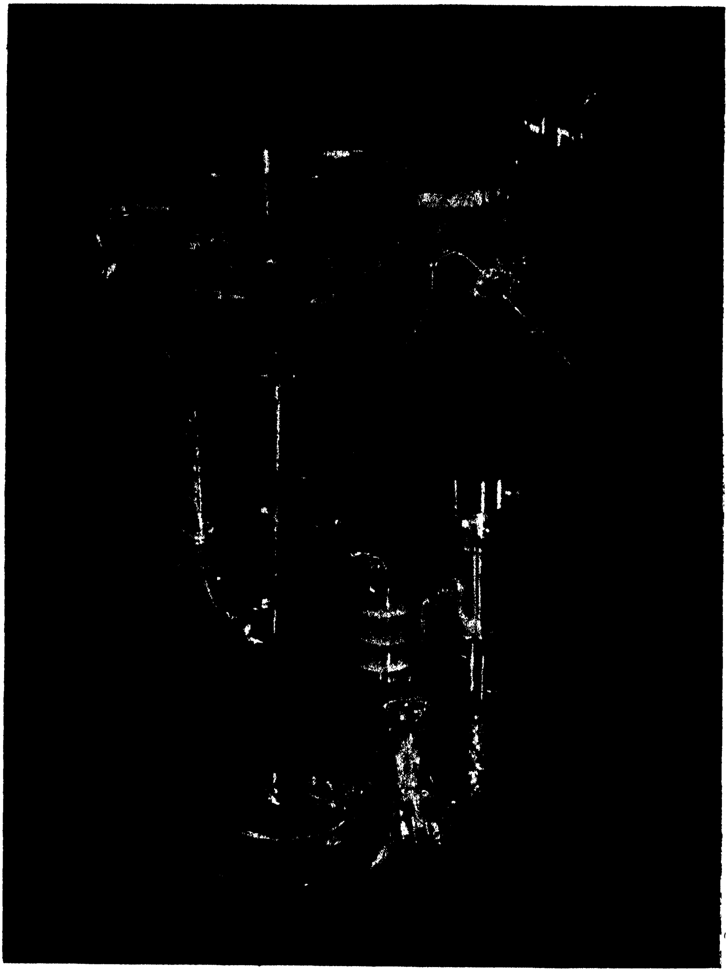

Fig. 3.—Showing the Thomas pump assembly. The double feed tubes are seen to the left entering the organ chamber; in this way the experimental and the control specimens can be cultivated at the same time in the same pump.

vascular bed is directly connected with the pump. After having passed through the capillaries the perfusion fluid will leave the organ by oozing from the numerous cut venules. This fluid is collected and filtered and passes into the equalization chamber where it is made to contact the gas mixture (80 p.c.

oxygen, 3 p.c. carbon-dioxide, and 17 p.c. nitrogen). From here the fluid is directed into the inferior chamber, the reservoir, whence an ascending tube carries it rhythmically back to the arterial side of the organ's capillaries. The pressure, the amplitude, and the frequency of the pulse can be easily regulated. Several pumps may be run simultaneously in the same incubator. Usually, double experiments are carried out, one organ being perfused under the experimental conditions in one pump while the control organ is cultivated in another.

The greatest care must be taken during the surgical dissection to secure the most perfect asepsis. The insertion of the organ into the culture chamber requires skilful handling because intrusion of air-bubbles into the artery must be strictly avoided. As soon as the organ has been safely placed in the pump and the perfusion has commenced, only occasional supervision of the apparatus is necessary.

In order to estimate whether the cultivation of an organ has been successful or not, the vitality of the tissue may be judged by one or more of the following criteria :—

1. The *physiological activity.* The function of the organ may be measured during the experiment in such cases where a specific secretion is yielded. Bing * found, for example, that a perfused pancreas could set free insulin in the circulating medium. Baker † found that iodine is set free by the thyroid gland during its cultivation in the Lindbergh pump and that the iodine is present in the medium in two forms: part of it is precipitated with the globulins, part is found in the protein-free filtrate. Carrel found that urea was concentrated by the kidney when perfused with 30 or 25 p.c. heparinized whole blood ; the functional state of the perfused kidney was, however, unsatisfactory.

2. The condition of the organ can be ascertained by *histological examination.* Samples of tissue may be secured during the cultivation or the organ may be fixed after the perfusion is finished. The completeness with which the artificial circulation has been carried through can be estimated quite effectively by examination of just ordinary preparations. The most common indication of partial circulatory deficiencies is the presence of small necrotic areas. The stainability of the nuclei may be altered as in the first stages of degeneration, owing to regressive changes of the parenchyma. Generally, the histological examination will reveal only the coarser alterations. It may be appropriate, therefore, to supplement the histological routine work with more refined methods.

3. *Cytological examination* is the most searching test to which the perfused tissue can be put. Presumably, neither mitochondria nor Golgi apparatus will be conserved unless the cells have retained a high degree of vitality. Likewise, the presence of mitotic figures is supposed to indicate that the tissue has remained in perfect condition.

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* Carrel and Lindbergh, " Culture of Organs," 1938, *loc. cit.*, p. 206.

† Baker, Lillian E., " The Secretion of Iodine by Thyroid Glands Cultivated in the Lindbergh Pump," *Science*, 1938, **88**, 479.

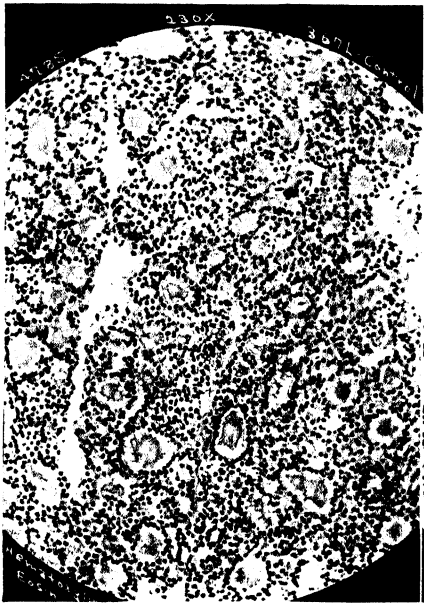

Fig. 4 (*a*).

Fig. 4 (*b*).

Fig. 6 (*a*).

Fig. 6 (*b*).

[*To face p.* 108.


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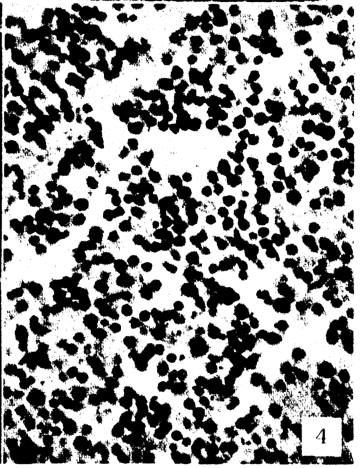
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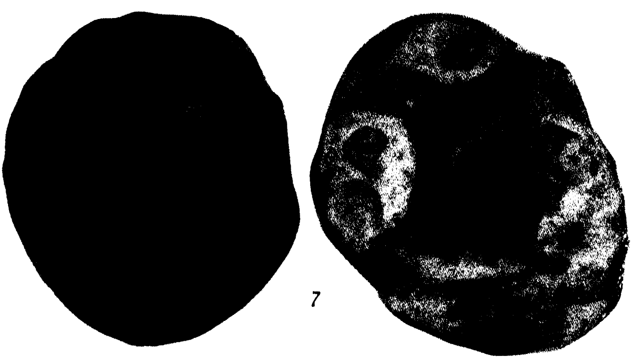
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4. *Explantation* of fragments after the termination of the experiment will, of course, show whether the tissue was dead or not. But the value of this test is restricted. Obviously, growth of fibroblasts may well be obtained even if the specific elements of the organ are necrobiotic.

The histology of different kinds of perfused organs varies greatly; some stand the cultivation better than others. The thyroid gland has been used more frequently than any other organ because its parenchyma sustains the artificial conditions remarkably well. If the culture medium is renewed several times, a thyroid gland may be cultivated for one month, at the end of which the histological aspect of the parenchyma is suggestive of decreased functional activity but otherwise quite normal (pl. I, fig. 4).

Some organs are extremely difficult to cultivate. Series of unsuccessful attempts were made before ovaries and testes could be perfused for only a couple of days without serious alterations. The ovaries stood cultivation better than the testes; even in the best conserved testes numerous spermatide giant-cells are formed in the tubules as a consequence of the artificial circulation. Some organs have not yet been successfully cultivated; the kidney, for example, shows profound morphological changes of the parenchyma after but one day's perfusion. Probably, the kidney's consumption of oxygen is so high that the Lindbergh pump is unable to cope with it.

Surprisingly, some organs pertaining to the nervous system have been able to stand cultivation better. Sympathetic ganglia remained histologically in almost normal condition after 12 days of perfusion; well-preserved neurofibrils have even been demonstrated after that period. On the other hand, lymphatic organs such as the spleen and lymph glands are badly altered by the cultivation (pl. II).

There can be no doubt that many types of organs remain alive and in almost normal condition after one week of perfusion. But the perfusion does alter the structure more or less and the specific elements do react a little differently—even in the case of the thyroid which seems to be the most resistant of glands to be cultivated. Before discussing the future of the method, it would be relevant, therefore, to add a few details to the reported cytological facts concerning the perfused thyroid gland. Mitochondria and Golgi apparatus can be demonstrated in the cultivated thyroid gland during the first few days of perfusion. The parenchyma is manifestly intact at the early stages of cultivation.* And the thyroid epithelium responds *in vitro* to stimulation with anterior pituitary extract in a manner closely resembling that observed in thyroids *in situ* (pl. I, fig. 6). But it should be noted that the perfusion alone tends to release a peculiar cellular reaction, a tremendous swelling of some of the epithelial cells during the first 1–2 days after the artificial circulation has been established. Only a few cells of any given vesicle react in this way, but the contrast between such cells and their

* Okkels, Harald, "The Effects of Perfusion on the Thyroid Epithelium," *J. Exp. Med.*, 1937, **66**, 297.

cuboidal and more deeply stained neighbours is usually very striking (pl. II, fig. 7). The swelling is not a degenerative phenomenon; it is more like the initial cytological reaction during increased secretory activity. The exact meaning of this heterogenous state of the epithelium is not yet known. It should be emphasized, however, that the phenomenon has been observed only in cultivated thyroid glands (fig. 8).

Another point should be noted. The very act of perfusion is supposed to give rise to a differential mechanism by which the activity of the thyroid epithelium must be influenced. Each time the circulating medium passes the gland a certain amount of active iodine compounds is secreted into the

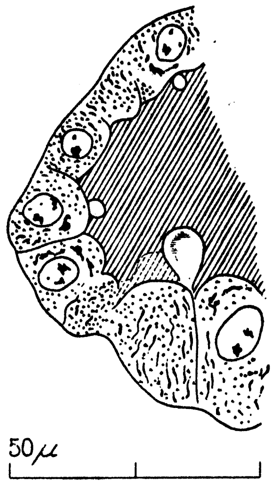

Fig. 8.—Rabbit's thyroid gland perfused for 24 hours. The Golgi apparatus in the swollen cells is dispersed in the cytoplasm.

fluid. In the absence of peripheral tissues these compounds are returned quantitatively to the gland, and it is hard to imagine how this iodine could avoid to react upon the parenchyma. This reaction should be a decrease of the cellular activity and an increased storage of colloid. Thus, what we observe by the cytological analysis of the cultivation experiments is the combined effects of (1) the perfusion itself; (2) the specific experimental intervention (e.g. addition to the medium of chemicals, hormones, vitamins, etc.); and (3) the differential reaction of both which may tend to counterbalance the desired experimental reaction.

The methods for culture of organs have been in existence only a few years. Many difficulties will yet have to be overcome and much research is required before the cultivation of organs can be utilized with advantage in experimental histology. The method will probably not be universally adopted by biologists for a long time; the complicated and expensive technique will

prevent that. The worst hindrance is the necessity of having a specially trained staff at disposal both in the operating room and for the supervision of the working pumps and auxiliary apparatus.

It would be rash, however, to conclude that the culture of organs is a method which, without being indispensable, can be operated only at prohibitive cost; that, in short, this ingenious technique is a white elephant. On the contrary, it would be unfair to underestimate the possibilities of the culture of organs. As a supplement to tissue culture cultivation of organized tissues will be of the greatest value for histologists and embryologists alike. Biochemical and endocrinological problems may be solved by utilizing the new culture method. And pathologists may advantageously adopt the perfusion apparatus; study on malignant growth, research on various infections, and work on virus problems are as many promising fields for the oxygenator pump.

DESCRIPTION OF PLATES.

PLATE I.

Fig. 4.—Cat's thyroid gland; left side (*a*) kept as a control and not cultivated. Right lobe (*b*) cultivated in 40 p.c. cat serum for a period of one month. Compared with the control the parenchyma of the perfused lobe is less active but otherwise in good condition. (Experiment made by Carrel, March, 1937.) Fixation, Zenker-formol. Hematoxylin-eosin. ×120.

Fig. 6.—Rabbit's thyroid gland; to the left (*a*) is the control lobe perfused for 52 hours with 40 p.c. serum; to the right (*b*) is the other lobe perfused for 52 hours with 40 p.c. serum plus 0·8 p.c. thyrotropic hormone. Fixation, Zenker-formol. Heidenhain iron-hematoxylin. ×350.

PLATE II.

Fig. 1.—*Colliculus oophorus* from cat's ovary perfused for three days with 50 p.c. serum.

Fig. 2.—Testicular tubule from rabbit perfused for 24 hours with 25 p.c. heparinized whole blood and 40 p.c. rabbit serum in Tyrode's solution. Note the spermatid giant cell.

Fig. 3.—Upper cervical sympathetic ganglion from cat. Perfused for twelve days with 40 p.c. cat serum. Neurofibrils are stained with Bodian's silver impregnation.

Fig. 4.—Lymph gland from cat. Perfused for two days with 40 p.c. serum. Histiolytic alterations.

(These experiments were all made by Carrel. We are indebted to Dr. Carrel, and to Paul B. Hoeber Inc., for permission to reproduce these figures and those reproduced in text-figures 1 and 2.)

Fig. 7.—Rabbit's thyroid gland. Two vesicles from two different glands showing heterogenous state of epithelium with swelling of single cells. Perfused for 24 hours. Fixation, Mann-Kopsch. Heidenhain iron-hematoxylin. ×850.

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VIII.—TUBE-LENGTH IN PHOTOMICROGRAPHY.

By John R. Baker, M.A., D.Sc.

(From the Department of Zoology and Comparative Anatomy, Oxford.)

When a microscope is focussed visually and then placed against a camera without any adjustment of focus, the object seen by the eye is not found to be in focus on the ground glass of the camera. If the eye which focussed the microscope was accommodated for distant vision, the plate would have to be placed at infinity to secure an image of the object focussed.

When high-power objectives are used, the thickness of the object (even when that is a thin section) is so great in relation to the depth of focus that some part of the object is usually found to be in focus on the ground glass; but the part focussed visually is not.

Two alternative methods are commonly employed to bring the image into focus. The first is to substitute a projection eyepiece for the ordinary one, and to change its focus until the image of the object seen visually is sharp. The second method is to move the fine adjustment of the microscope, and thus shift the position of the real image formed between the lenses of the eyepiece towards the field-lens. The eye-lens now receives the rays from any point on the real image at a different angle, and instead of refracting them to infinity causes them to form a second real image on the ground glass.

The use of a projection eyepiece is satisfactory for many purposes, but high power eyepieces of this type are not made, and it is therefore necessary to use a long camera if high magnification is required. Not every microscopist has a projection eyepiece, and they are expensive. Further, to get perfect results it would be necessary to own one ordinary and one compensating projection eyepiece, and two types are not made. The alternative method is not satisfactory for really critical work, although it is very commonly employed; for the objective is only spherically corrected when at a certain fixed distance from the object, and to change this distance is to disturb its corrections.

In this paper a third alternative method is suggested, for which advantages are claimed.

Every part of this paper refers to the photomicrography of objects mounted in balsam or other medium of similar refractive index and covered by coverglasses of the thickness for which the objectives are corrected. The expression "tube-length" is used to mean the distance between the shoulder

of the objective and the end of the tube (i.e., it includes the thickness of nosepiece or other objective-changer). My own objectives are corrected for 160 mm., and this will be taken as the standard in what follows, though the method is applicable also to objectives requiring other tube-lengths, provided that all one's objectives require the same length. The general description of the method refers to the Huyghenian eyepiece, but the method works equally well with other types. The description applies to the horizontal camera, but the method is applicable also to vertical cameras. When a ground glass is referred to, the remarks are equally applicable to a plane glass focussing screen on which the image is made visible by the use of a lens.

The problem is to produce on the photographic plate an image of the object focussed visually without changing the eyepiece and without upsetting the corrections of the objective by altering its focus. This is easily achieved by increasing the tube-length. This brings the real image formed within the eyepiece nearer to its lower or field lens, so that the eye-lens forms a second real image on the ground glass or plate. The focussing of the microscope for photomicrography, then, is done by altering the tube-length and leaving the fine adjustment alone. It is essential to have a smoothly-working tube, to make sure that the position of the objective is not changed when the tube is drawn out, but a rackwork to the tube is not necessary.

Accurate focussing of minute detail on a ground glass screen is very tiring work. If the focussing be done, as here suggested, by altering the tube-length, the focussing of the image in the camera can be entirely dispensed with by following the procedure described below.

The board which holds the microscope against the camera must be provided with sockets which ensure that the distance between the stage of the microscope and the plate is always the same. This distance is 46·5 cms. in my own set-up, but others who prefer a longer camera can make the distance whatever they like.

The microscope, in a horizontal position, is focussed visually with great accuracy, the tube-length being 160 mm. The miscroscope is now gently lifted and placed in its sockets against the camera. The tube is very carefully lengthened until the part of the object which was exactly focussed visually is sharply in focus on the ground glass. The amount by which the tube was lengthened is now noted. The procedure is repeated several times, until a reliable figure is obtained. This figure is then recorded. With my own camera-length the figure for a Watson $\times$ 5 Huyghenian eyepiece is 13 mm. This figure is independent of the objective used (provided that the objectives are roughly parfocal, so that the effective camera-length is not altered when one changes from one objective to another). It is convenient to use a 16 mm. or 4 mm. objective to find what extension of the tube is necessary when passing from visual observation to photomicrography. It will be found that the same extension is required when the oil-immersion 2 mm. objective is used with the same eyepiece.

When the distance between the microscope stage and the plate is 46·5 cms.,

the necessary extensions of the tube with the eyepieces which I use are as follows :—

Eyepiece.	Extension of tube beyond 160 mm.
Watson. Holos. × 14	$1\frac{1}{2}$ mm.
Zeiss Compens. × 12	2 „
Watson Holos. × 7	8 „
Watson Huyghenian × 5 ..	13 „

With longer cameras the necessary extensions of tube-length are less.

(It may be recalled that the Watson Holos. eyepieces have little draw-tubes of their own, whose extension transforms them from ordinary to compensating eyepieces. With the Holos. × 14 eyepiece, the extension of the microscope draw-tube on changing from visual observation to photomicrography remains $1\frac{1}{2}$ mm., whether the eyepiece is being used as an ordinary or a compensating one, and similarly the extension is 8 mm. with the Holos. × 7 under both circumstances.)

When the necessary extensions have been carefully ascertained for each eyepiece that one uses, the ground-glass focussing screen may be discarded, as it will not be required again. The only focussing necessary in future will be visual focussing. Directly some object is noticed which one wishes to photograph, the microscope is put in the horizontal position and the tube-length is adjusted to exactly 160 mm. (if it is not already at that figure). The part of the object which it is desired to record is now carefully focussed with the fine adjustment. The tube of the microscope is next very gently extended to a distance which depends on the eyepiece in use, and which will already have been determined and recorded. The microscope is then gently lifted and placed in its sockets against the camera. The same colour-screen is used as was used in visual focussing. A plate is inserted in the camera and the exposure is made without any focussing on a ground glass.

It is important that the source of light for photomicrography should be at the same distance from the microscope as that used in visual work, so that it is unnecessary to change the focus of the condenser. This is achieved by fixing the photomicrographic lamp immovably in such a position that when the microscope is placed in the sockets the distance between the source of light and the back lens of the condenser is 250 mm. (as in visual work). Needless to say, the source of light is fixed once and for all so that it is in line with the optical axis of the microscope and camera.

Powerful lamps are usually used in photomicrography, partly to shorten the exposure but mainly to give sufficient light for focussing on the ground-glass screen when high magnification is needed. In the method here suggested no such focussing is done, and an ordinary pearly-surface electric bulb gives plenty of light for a reasonably short exposure. A 60-watt Cryselco coiled-coil bulb is convenient.

The method described in this paper is almost as quick and easy as the use of an attachable eyepiece-camera. The latter is convenient, but does not provide photographs showing the ultimate detail of which the microscope is

capable. The method described here gives the best results which the microscope can produce by any means. The focussing is particularly exact. An example may be quoted. The early spermatids of the newt (*Triturus vulgaris*) contain a ring-centriole and a dot-centriole, which lie close together. In favourable circumstances one sees a figure resembling an o with a dot within it. In one such case I found that a movement of the very delicate fine adjustment of the Watson Edinburgh Student's H microscope through a circumferential distance of only 1 mm. sufficed to cause the appearance to change from out of focus on one side to out of focus on the other (using oil-immersion 2 mm. objective and Holos. $\times$ 14 eyepiece). A movement of the circumference of the fine-adjustment wheel through 1 mm. represents a change of focus of less than 1μ. Nevertheless, good photographs of the object were taken by the method described in this paper, showing the ring and the dot within. The free border of the intestinal epithelium of Vertebrates, with its so-called striations, is another very difficult subject for photomicrography. All the photographs illustrating my paper on this subject (Baker, 1942) were taken by the method here described.

A microscopist who decides to use this method will have to fix his camera-extension, make sockets to hold his microscope, and clamp his photomicrographic lamp in position. He will then have to find for each of his eyepieces the distance by which the tube-length of the microscope must be extended on passing from visual observation to photomicrography. This is rather a tedious job, if it is done accurately, as it must be. Once it is done, however, it is done for ever. From that moment onwards photomicrographs can be taken with surprising ease, accuracy and absence of fatigue. All the fiddling about which accompanies ordinary photomicrography is eliminated, and one has the satisfaction of knowing that one's objectives are always being used at the exact distance from the object for which they are corrected. There is thus no deterioration of image in passing from visual to photomicrographic work.

Summary.—It is recommended that focussing in photomicrography should be achieved by extending the draw-tube, the other adjustments of the microscope being left exactly as they were during visual observation. The amount of extension necessary depends wholly on the eyepiece, and once it is known, the ground-glass may be discarded. On passing from visual work to photomicrography one simply extends the tube by the amount which the eyepiece used is known to require.

It is claimed that this method is far quicker, easier, and less fatiguing than the ordinary method in which the object is focussed on a ground-glass screen. It gives critical images at all magnifications, because the objective remains at the distance from the object which its corrections require.

REFERENCE

Baker, J. R. (1942).—Quart. Journ. Micro. Sci., in the press. "The free border of the intestinal epithelial cell of Vertebrates."

ABSTRACTS AND REVIEWS.

ZOOLOGY.

(*Under the direction of* G. M. FINDLAY, M.D.)

HISTOLOGICAL TECHNIQUE AND STAINING.

The Chromatophores of Fundulus heteroclitus in Polarized Light.—A. M. SHANES and R. F. NEGRELLI (*Zoologica.*, **26**, 237). Granular material associated with the melanophores on the scales of *Fundulus* shows birefringence of varying intensity, at an intermediate stage between zero and maximum birefringence the granules show Brownian movement. They appear to be identical with the granules of the xanthophores; they occur in masses with anastomosing processes in which they move freely. Movement of melanin granules in the melanophores appears to be intimately associated with this doubly refracting material. They react to adrenalin, ether, and KCl, as do the melanophores. J. A. M.

Dye Adsorption by Bacteria at Varying H-ion Concentrations.—T. M. MCCALLA and F. E. CLARKE (*Stain Technol.*, 1941, **16**, 95). Adsorption of H-ions and dye cations by washed bacterial cells shows a reciprocal relationship. Apparently H-ions and crystal violet ions are held by the cell at the same adsorption centres, and the influence of H^+ on basic dye adsorption is one of direct competition or replacement. The adsorption of H^+ and acid fuchsin is similar in that an increase is noted as the pH of the suspension is lowered. (Author's abstract.)

Plant Virus Differentiation by Trypan-blue Reactions within Infected Tissue.—F. P. MCWHORTER (*Stain Technol.*, 1941, **16**, 143). Trypan-blue has proved effective for demonstrating the presence of certain plant viruses within infected tissues. The amorphous and crystalline inclusions which constitute cytological evidence of viruses stain proportionately. The effects produced by different viruses react differently to the stain and those inclusions which do not absorb trypan-blue tend to stain with phloxine. This selective staining is the basis for using trypan-blue singly and in combination with phloxine as standardized procedures for demonstrating and differentiating cytological evidence of plant viruses. These tests are very rapid and are especially applicable to temporary mounts of living tissue, but permanent mounts can be made from material fixed in formalin. (Author's abstract.)

The Germination and Staining of Basidia in Gymnosporangium.—L. S. OLIVE (*Stain Technol.*, 1941, **16**, 149). Spores of *Gymnosporangium* (a rust on red cedar) are germinated on the slide in a damp chamber (*circa* 3 hours). Fix by inverting over osmic acid (4–5 minutes). Allow to dry which fixes them to slide with the gelatin produced from stalks during germination. Dehydrate thoroughly (one or more hours in higher alcohols). Return to water: mordant 2–3 hours 4 p.c.

iron alum; stain 2–3 hours 0·5 Heidenhain hæmatoxylin; destain in 2 p.c. iron alum. Dehydrate very thoroughly in alcohols; xylol-absolute (1 : 2, 1 : 1, 2 : 1) xylol; balsam. (Author's abstract) abbreviated.

Permanent Stained Preparations of Thick Blood Films.—W. D. Gingrich (*Stain Technol.*, 1941, **16**, 159). Spread large drop of blood on slide over area 1 cm. diameter, allow to dry, stain and lake in dilute Giemsa, rinse in water, allow to dry. Flood with May-Grünwald (0·5 p.c. in methyl alcohol) for 30 seconds, rinse in water, allow to dry. (MacNeal's tetrachrome in methyl alcohol and glycerin may be substituted for Giemsa and solution in methyl alcohol instead of May-Grünwald.) Mount in Diaphane. (Author's abstract) abbreviated.

Preparing Permanent Deep Chamber Mounts of Variable Dimensions.—D. L. Bassett (*Stain Technol.*, 1941, **16**, 165). Aluminium wire, gauge 14–24 (1·63–0·51) is wound tightly on a cylinder or square rod of coverglass diameter less twice the thickness of the wire used, as many turns as cells wanted. Slide off carefully and cut with scissors. Shape each individually in a small smooth-faced vice so that they will lie flat on slide. Centre wire on slide, transfer tissue to chamber, fill up with Clarite (70 p.c. in toluol) and apply coverglass, let down carefully from one side after teasing out any air bubbles from specimen. The crust which forms on surface of toluol-Clarite dissolves when coverglass is so applied. J. A. M.

Acetic-orcein: A New Stain-fixative for Chromosomes.—L. La Cour (*Stain Technol.*, 1941, **16**, 169). Standard solution 1 p.c. orcein in 45 p.c. acetic acid, increased in special cases (Drosophila) to 2 p.c. orcein in 70 p.c. acetic. Root tips addition of 1 c.c. HCl to 10 c.c. standard solution assists maceration. Permanent mounts—separated slide and coverglass passed through 80 p.c. alcohol 2 minutes. Absolute 2 minutes, 2 changes cedarwood oil, 5 minutes each. Replace coverglass, mount in thickened immersion oil or balsam. J. A. M.

A New Microchemical Reaction for Cellulose.—E. E. Post and J. D. Laudermilk (*Stain Technol.*, 1942, **17**, 21). Two solutions required: (1) 2 p.c. solution of iodine in 5 p.c. KI, diluted with 9 parts water containing 0·28 p.c. glycerin; (2) saturated solution of LiCl in water. Apply 2 or 3 drops of (1) with a glass rod; allow preparation to stand for 30 seconds; blot with filter paper, drying as completely as possible. Apply 1 drop of (2), cover, and examine. Colour reaction will be obtained within 5 minutes. Pure cellulose light blue. The reaction has been used successfully for plant histology. J. A. M.

Flagella Staining of Anærobic Bacilli.—E. O'Toole (*Stain Technol.*, 1942, **17**, 33). For the elaborate details necessary for staining flagella of anærobes consult original. Good photomicrographs. J. A. M.

Notes on the Sanitary Importance of the Simulidæ and on their Internal Morphology.—L. Vargas (*Rev. Inst. Health and Tropical Diseases (Mexico)*, 1941, **2**, 213). Review of importance of Simulidæ as vectors of diseases of domestic animals and description of internal anatomy, with many figures, line drawings, and photomicrographs. J. A. M.

Diplopoda from Southern India and Ceylon: Part 2, Neuratophora and Juliformia.—J. Carl (*Rev. Suisse de Zool.*, 1941, **48**, 569). Ecology and classification with detailed diagnostic description of species. Beautiful line drawings of exoskeleton and appendages. J. A. M.

Histological Studies of the Chick Lung in Later Embryonic Stages (in German).—S. Harada (*Okaj. Folia anat. jap.*, 1939, **18**, Pt. 6). Chick embryos from the 12th to 21st day of incubation were examined. By the 20–21st day the

chick has begun to breathe through shell perforation and the adult condition is attained. The alveolar lining consists of epithelial cells continuous with the lining of the "lung-pipes" corresponding to the mammalian bronchioli. Good figures on two coloured plates. J. A. M.

Pure Culture of the Hepatic Cancer Tissue produced by Dimethylaminoazobenzene.—S. Morigami (*Jap. Jour. Med. Sci., V., Pathology*, 1939, **4**, No. 2). Liver tumour produced in a rat by feeding dimethylaminoazobenzene was cultured through many generations *in vitro*, a pure culture of the epithelial cells being obtained after 5 or 6 generations. By placing a fragment of normal tissue in the same drop of medium the growth of the carcinoma was improved. The pure cultures implanted into rats gave rise to tumours identical with the original. J. A. M.

On the Connection between the Bile-capillaries and the Bile Ducts in the White Rat (new injection method).—S. Omoti and I. Kitayama (*Okaj. Folia anat. jap.*, 1939, **18**, Pts. 4–5). Injection of 10 p.c. solution of "Kernilin" into common bile duct and 2½ p.c. iron alum solution into portal vein leads to formation of an insoluble black precipitate in the bile ducts and capillaries. After fixation in formol (10 p.c.) paraffin serial sections are stained hæmatoxylineosin. The transition is abrupt, capillaries sometimes having ductepithelium on one side and liver cells on the other. J. A. M.

Studies on the Elementary Body of the Polyhedral Disease of the Silkworm.—T. Tanigouchi, M. Asano, M. Hosokawa, and K. Ishikawa (*Jap. Jour. Exp. Med.*, 1939, **17**, No. 4). Adult larvæ or young pupæ were inoculated, or larvæ infected by feeding. When symptoms appeared the blood was examined, and if polyhedral bodies, but no bacteria, were present, films were prepared from the fat bodies, dried, and stained. Fine granules (0·1–0·15μ) and polyhedral bodies are seen, the latter inside the nuclei. No evidence could be obtained that the minute elementary bodies were contained in the polyhedral bodies, although it is probable that the E.B. also occur inside the nuclei. Special staining preliminary mordant 1 p.c. aq. eosin after fixation of the dried films with methylalcohol-formol-acetic. The actual staining solution contains Eosin-methylen blue, Azur 1, crystal violet in methylalcohol-glycerin ripened for 2 weeks. J. A. M.

The Histochemical Demonstration of Hæmoglobin in Blood Cells and Tissue Smears.—P. H. Ralph (*Stain Technol.*, 1941, **16**, 105). Dried blood film or tissue smear is fixed by flooding with 1 p.c. solution of benzidine in absolute methyl alcohol for 1 minute. The benzidine solution is poured off and replaced by solution of superoxol 25 p.c. in 70 p.c. ethyl alcohol. Stand for 1½ minutes, wash in Aq. dest. 15 seconds, dry, and mount in neutral Canada balsam. All structures containing hæmoglobin are coloured dark brown. Counter stain if required with Wright's stain. J. A. M.

Protozoa

Cultivation of Protozoa.—L. H. Hyman ("Lettuce as a medium for the continuous culture of a variety of small laboratory animals," *Trans. Amer. micr. Soc.*, 1941, **60**, 365–370). The use of lettuce as a basic medium is recommended for different small animals, including protozoa. Lettuce is placed in an aluminium container with water and brought to a boil. Boiled leaves may be stored in a refrigerator until required. Raw lettuce is said to be unsuitable for culture purposes. Protozoa cultivated with lettuce include *Paramecium* and hypotrichous ciliates, small flagellates, amœbæ, and other Rhizopods. C. A. H.

Protozoa from Ground Squirrel.—M. Tanabe and M. Okinami ("On the parasitic protozoa of the ground squirrel, *Eutamias asiaticus uthensis*, with special reference to *Sarcocystis eutamias* sp. nov.," *Keizyo J. Med.*, 1940, **10**, 126–34, 3 pls.).

The author found various protozoal parasites in the ground squirrel, *Eutamias asiaticus*, from Japan. One of these animals was infected with a Sarcosporidian, *Sarcocystis eutamias* sp.n., while others harboured the following intestinal forms: *Entamœba citelli*, *Trichomonas muris* var. *citelli*, *Pentatrichomonas eutamias* sp.n., *Pentatrichomastix eutamias* sp.n., *Chilomastix magna*, *Hexamita eutamias* sp.n., and *Eimeria beecheyi*. All these forms are described and illustrated. C. A. H.

Simian Amœbæ.—H. Salis ("Studies on the Morphology of the *E. histolytica*-like Amœbæ found in Monkeys," *J. Parasitol*, 1941, **27**, 327–41, 2 pls.). Description of intestinal amœbæ found in twelve species of monkeys. The parasites belonged to two species: *E. histolytica*, which was found to be indistinguishable morphologically from the same organism in man; and *E. chattoni* representing a non-pathogenic species peculiar to monkeys. The latter amœba is uninucleate both in the active and encysted stages, and includes two size races. Some strains are discernible by their characteristic chromatoid bodies. C. A. H.

Development of Pandorina.—C. E. Taft ("Inversion of the developing cœnobium in *Pandorina morum* Bory," *Trans. Amer. micr. Soc.*, 1941, **60**, 327–8). The author has demonstrated that, as in the case of other Volvocidæ, *Pandorina* undergoes inversion in the course of its development. The plate is first curved, but later becomes flat and inverted, until the corners meet to produce a hollow spherical cœnobe. C. A. H.

Flagellate Infection of Termites.—E. May ("The Behavior of the Intestinal Protozoa of Termites at the time of the last Ecdysis," *Trans. Amer. micr. Soc.*, 1941, **60**, 281–92). Observations were made to determine the method of refaunation of termites (*Zootermopsis* and *Kalotermes*) with intestinal flagellates at the time of the last moult preceding the adult stage. It was found that before this takes place the protozoa become reduced in numbers and in size, owing to scarcity of food, but some of them are confined within the shed intima, whence they are released into the new gut and serve to reinfect the adult insect. No cysts of any of the flagellates have been observed. C. A. H.

Trichomonad of Frog.—R. Samuels ("The Morphology and Division of *Trichomonas augusta* Alexeieff," *Trans. Amer. micr. Soc.*, 1941, **60**, 420–40, 4 pls.). An account is given of the cytology and process of multiplication in the frog trichomonad, *T. augusta*. In addition to the structures usually observed in these flagellates, a "ring-like extranuclear, granular cloud" is described. Particular attention is drawn to variation in cytological details, as seen after fixation and staining by different methods. The process of division, including the behaviour of various internal organellæ, is described in great detail. C. A. H.

Trichomonad of Cat.—M. Tanabe ("Notes on the Morphology of *Pentas trichomonas felis* from the Cat," *Keizyo J. Med.*, 1940, **10**, 124–5, 1 fig.). Description of the flagellar apparatus in the intestinal flagellate of the cat, *Pentatrichomona-felis*. There are usually five anterior flagella, of which four start from a large blepharoplast (together with the axostyle, basal rod, and undulating membrane) and are directed forwards, while the fifth, originating from a small blepharoplast, trails back. C. A. H.

Effect of Heat on Plasmodium.—F. Coulston ("Thermal Death-point of the

Erythrocytic stages of *Plasmodium circumflexum*," *J. Parasitol*, 1941, **27**, 265–6). The reaction of the avian malaria parasite, *Plasmodium circumflexum*, to heat was studied by placing infected blood in a constant temperature incubator equipped with a De Khotinsky thermal control. The thermal deathpoint of the parasite was found to be 50° C. after exposure for 15 minutes, and 55° C. after 5 minutes. The parasites undergo little morphological change, but tend to round up and stain deeper with Giemsa's stain, and there is no hæmolysis of the blood. C. A. H.

Ciliate from Horse.—T. G. Davis (" Morphology and Division in *Tetratoxum unifasciculatum* Gassovsky," *Trans. Amer. micr. Soc.*, 1941, **60**, 441–52, 3 pls.). This paper provides a revised description of an oligotrichous ciliate, *Tetratoxum unifasciculatum*, normally found in the colon, but occasionally also in the cæum, of the horse. During division the micronucleus behaves in the same manner as in other ciliates, but a definitive equatorial plate is absent in the metaphase stage. The number of chromosomes appears to be 14. C. A. H.

Foraminifera from Lower Lias of Dorset.—W. A. Macfadyen (" Foraminifera from the Green Ammonite Beds, Lower Lias, of Dorset," *Phil. Trans. R.S. Lond.*, Series B., 1941, No. **576**, 1–73, pls. 1–4, text-figs. 1–6). This is the most important paper on British Lias Foraminifera published within the last three-quarters of a century, and is the more welcome as the author has avoided the multiplication of trivial names adopted by most authorities. He points out that such numerous forms are " generally ill-defined and insufficiently differentiated from neighbouring species both in nature and by their authors. A natural consequence of this is that the greater the number of such similar species that are proposed the more do intermediate specimens become unnameable. . . . It is preferable to subdivide these ill-defined aggregates of a population rather broadly, using few trivial names, and allowing considerable variation to each species. Even so it is sometimes a matter of opinion as to what name to use for a given specimen. This view is supported by the variability which is found among a group of specimens in a single sample, so that neither peculiar geographical distribution nor time change can be cited in favour of splitting (p. 6)." If only such commonsense views had been followed in recent years by other authors what endless confusion would have been prevented !

Fifty-five species, with full synonyms, are described and figured. They are referred to twenty genera and six families, of which no less than eleven genera and forty-five species belong to the family Lagenidæ which predominate in the material. " In this family there appears to be a wide variation within some of the groups where neither species nor even genera are sharply defined."

One new genus *Carixia* (genotype *C. langi*) and one new species *Lagena davoei* are defined, and two new names are proposed to replace invalid names. These are *Lingulina terquemi* = *Frondicularia rhomboidalis* Terquem 1862, Terquem's specific name being preoccupied by d'Orbigny ; and *Tristix*—a new generic name for those hyaline forms consisting of a number of chambers, generally triangular in section, joined in a straight series with a simple terminal aperture. Such forms have in the past been incorrectly referred to *Triplasia* Reuss or *Rhabdogonium* Reuss.

The new genus *Carixia*, tentatively assigned to the family Opthalmidiidæ, has an adherent reticulation of unsegmented, imperforate, calcareous tubes set in a groundwork of calcareous cement ; the apertures are simple at the open ends of the tubes, but the early development of the test is unknown. A. E.

Caribbean Foraminifera.—J. A. Cushman (" Recent Foraminifera from Old Providence Island collected on the Presidential Cruise of 1938," *Smithson. Misc. Coll.*, 1941, **99**, No. 9, 1–14, pls. 1–2). There are no previous records from this

particular area but the fauna appears to be typically West Indian. Fifty-six forms are listed, and some of the more interesting are figured. Nearly all the species are among those described by d'Orbigny in his Cuban monograph (1839). Most of the others have a wide distribution in Indo-Pacific areas, a few have world-wide distribution. There are no novelties. A. E.

Dentalina and Nodosaria in American Upper Cretaceous.—J. A. CUSHMAN ("American Upper Cretaceous Foraminifera of the Genera *Dentalina* and *Nodosaria*," *Cont. Cush. Lab. For. Res.*, 1940, No. **223**, 75–96, pls. 13–16). The species of these two genera have been much confused in American Cretacous records —as elsewhere. Some have long, and others relatively short, vertical ranges, and are therefore good index fossils. A study of European types and topotype material has led to many corrections of nomenclature. More than twenty species and varieties of each genus are described and excellently illustrated. The paper will be of great value to Cretaceous workers in this country as well as in America, the foraminiferal faunas being largely identical. A. E.

Venezuelan Fossils.—J. A. CUSHMAN and H. H. RENZ ("New Oligocene-Miocene Foraminifera from Venezuela," *Cont. Cush. Lab. For. Res.*, 1941, No. **224**, 1–27, pls. 1–4). The Agua Salada formation of Venezuela is of Upper Oligocene to Middle Miocene age, and though lithologically uniform, can, according to the authors, be subdivided into seven distinct zones marked by the distribution of the smaller Foraminifera. The paper describes and figures 42 new species and 12 new varieties of Foraminifera, but the majority seem to be very like some old friends with new faces (or names). A. E.

Aguayoina is Cœlenterate not Protozoan.—P. J. BERMUDEZ ("Note on *Aguayoina asterostomata* Bermudez," *Cont. Cush. Lab. For. Res.*, 1941, No. **225**, 28). The genus *Aguayoina* discovered and described in 1938 by Bermudez, who referred it to the family *Saccamminidæ*, can no longer be classed with the Foraminifera. It is unquestionably a Cœlenterate and has been tentatively assigned by specialists in that phylum to the order Zoanthidæ. Its further relationships must be left to the study of living material A. E.

Variation in Bolivina.—J. A. CUSHMAN and RUTH TODD ("Statistical Studies of some *Bolivinas*," *Cont. Cush. Lab. For. Res.*, 1941, No. **226**, 29–31, pls. 5–8). The differences between numbers of specimens of (1) *Bolivina subœnariensis* Cushman, as it occurs in two different localities and environments at the present time; and (2) of *Bolivina marginata* Cushman and its accompanying variety *multicostata* Cushman as they developed and died out in one locality throughout several successive zones of the Florida Miocene, have been worked out and illustrated by graphs. From these preliminary studies the authors suggest the possibility that average measurements of a fair number of specimens may be useful as an indicator of relative age, where the range of a species is relatively long and an entire series can be studied. But they admit that changes of environmental conditions in fossil series may also have influenced size characters, and that much more work is necessary before any definite proof of the value of size-variation for long-distance correlation can be considered practicable. A. E.

Alaskan Fossil Foraminifera.—J. A. CUSHMAN ("Some Fossil Foraminifera from Alaska," *Cont. Cush. Lab. For. Res.*, 1941, No. **227**, 33–8, pl. 9). Material from a raised beach near Nome, Alaska, of uncertain but probably Pleistocene or Pliocene age, has yielded nearly twenty species of Foraminifera, nearly all of known Arctic range, though a few extend down the Eastern Pacific coast and several occur in the Pleistocene or Pliocene of both Atlantic and Pacific coasts. The characteristic

Arctic genus *Elphidiella* is most conspicuous and abundant and supplies the only new species *E. nitida*. E. A.

Species of Globigerina.—J. A. CUSHMAN ("The Species described as *Globigerina* by d'Orbigny in 1826," *Cont. Cush. Lab. For. Res.*, 1941, No. **228,** 38–42, pls. 10, 11, and 12, figs. 1 *a*, *b*, *c*). Twelve species were assigned to this genus by d'Orbigny in 1826, three of which were referred by him to Soldanian figures. Of the other nine only the habitat was given without further description, the author's drawings remaining unpublished until Fornasini reproduced outline tracings of them over seventy years later. The twelve species are discussed, and those which can be definitely recognized as *Globigerina* illustrated from topotype material on pl. 10. Fornasini's outline drawings from the "Planches Inédites" of d'Orbigny are reproduced on pls. 11–12. Several of d'Orbigny's species are referable to other genera or are unidentifiable. A. E.

Miocene Species of Uvigerina.—J. A. CUSHMAN and RUTH TODD ("Species of *Uvigerina* occurring in the American Miocene," *Cont. Cush. Lab. For. Res.*, 1941, No. **229,** 43–52, pls. 12, figs. 2–11, and pls. 13, 14). A well-illustrated study of twenty-three species and varieties of *Uvigerina* which have been recorded by various authorities from Miocene formations in America. There are no novelties. A. E.

American Cretaceous Cristellarians.—J. A. CUSHMAN ("American Upper Cretaceous Foraminifera belonging to *Robulus* and related genera," *Cont. Cush. Lab. For. Res.*, 1941, No. **230,** 55–69, pls. 15, 16). An excellently illustrated study of some species of a group which is abundant throughout the Coastal Plain Cretaceous strata of America, as also in contemporary European deposits. The author refers them to the genera *Robulus*, *Lenticulina*, and *Planularia*, but evidently appreciates the difficulty of separating forms (long recognized under the comprehensive generic name *Cristellaria*), for he admits that we have not sufficient data to know what allowances should be made for variation in a group where the microspheric and megalospheric forms show very considerable differences. A. E.

Fossil Uvigerinæ.—J. A. CUSHMAN and RUTH TODD ("Notes on the Species of *Uvigerina* and *Angulogerina* described from the Pliocene and Pleistocene," *Cont. Cush. Lab. For. Res.*, 1941, No. **231,** 70–8, pls. 17–20). A well-illustrated study of all the species of *Uvigerina* and *Angulogerina* which have been recorded from Pliocene or Pleistocene formations throughout the world. Two new species are described and figured: *U. juncea* from the Pliocene of Timm's Point, California, and *U. rutila* from the Pliocene of Algiers and several southern European countries. A. E.

A New Genus.—DAN E. FERAY ("*Siphonides*, a New Genus of Foraminifera," *Jour. Palaeont.*, 1941, **15,** No. 2, 174–5, figs. 1–4). *Siphonides* (genotype *S. biserialis*) occurs in the Weches formation, Claiborne group of the Middle Eocene at Southville, Texas. The diagnostic character of the genus is the biserial arrangement of the later chambers following an early and typical siphonine coil. The typical *Siphonina* aperture is maintained throughout, but occupies an eccentric position on the ventral side and close to the periphery of each of the biserial chambers. The test is very small. The genus represents a specialized advance in the phylogeny of *Siphonina*, subfamily Siphonininæ of the family Rotaliidæ. A. E.

Deep Sea Cores.—J. A. CUSHMAN ("A Study of the Foraminifera contained in Cores from the Bartlett Deep," *Amer. Jour. Sci.*, 1941, **239,** 128–47, pls. 1–6, text-figs. 1–10). Three cores taken with the Pigott sounding apparatus in the vicinity of the Bartlett Deep between Cuba and Jamaica show very decided changes in fauna during the time represented by their deposition. The region is one of known

gravity anomalies and might be supposed to have been relatively free from radical changes during the glacial periods. The cores were :—

No.	*Length of Core.*	*Depth.*	*Locality.*
P. 135	44 inches	2680 fathoms	Midway Jamaica–Cuba
P. 136	76 „	2540 „	To W. of Jamaica
P. 137	75 „	2675 „	To S. of Cuba, near Cayman Ids.

Preliminary examination of the cores indicated changing conditions marked by variation of colour and relative coarseness, but each contained an outstanding zone of very pure *Globigerina* ooze, though at different levels. Samples of approximately equal size taken from each core at these distinctive points were washed, and the residues showed marked differences in volume, except in the case of the *Globigerina* ooze samples, which yielded approximately the same volume of residue as the original samples, the Foraminifera being in an exceptionally perfect state of preservation.

Well-preserved Pteropod shells were found in some samples, particularly in core P. 135, which also yielded some Gorgonia spicules and a large number of relatively shallow water Foraminifera. Now Pteropods usually disappear by solution at about 1000 fathoms and *Globigerina* is seldom well preserved below 2000 fathoms, while Gorgonids are of comparatively shallow habitat. So the question arises whether these varying samples of each core were actually deposited at the great depth from which they were obtained, or whether changes of depth had been rapid in the area subsequent to the time of deposition.

If the cores were deposited at approximately the depth from which they were recovered, the time intervals represented by the entire cores must be relatively long. Schott has estimated the rate of deposition of *Globigerina* ooze as varying between 0·53–2·13 cm. per 1000 years, which would give an age of about 90,000 years at a mean rate and several times as long at the minimum rate.

A study of the pelagic species of Foraminifera considered in the light of what is known of the same species in this area to-day, indicates two periods of great abundance in core P. 137 and four periods in core P. 136, while core P. 135 shows far less evidence of rapid change of conditions. It may therefore be assumed that the area of P. 135 was largely outside the influence of the warm surface currents which influenced the abundant deposition of pelagic species in cores P. 136–7.

The benthic Foraminifera occur in relatively small numbers but are of interest because the majority have not been recorded from such depths as the cores represent. They are more frequent in P. 135 and the number of benthic species in this core is far greater than in the others in spite of its lesser length. Nearly all were found in one sample of this core and some were not recorded elsewhere. The author gives details of the known distribution of all the benthic species recorded and describes the structure of each core at length.

From the evidence the author concludes that there have been very decided changes in the area during the period of deposition, which may have been anything between 90,000–200,000 years, or even more. They include rhythmic changes of either colder and warmer surface water temperatures, or alternating periods of presence or absence of warm currents like the present Gulf Stream. These are evidently due to recurring climatic changes, perhaps coincident with a change of sea level. The changes may be coincident with known glacial and interglacial periods taking the times of deposition into consideration. There is also evidence that the depth may have changed appreciably at intervals during deposition.

A very interesting paper which, while adding to our knowledge of the conditions of oceanic deposition, reveals also how much more we shall have to learn to obtain even an outline of knowledge.

A. E.

Upper Oligocene of Cuba.—DOROTHY K. PALMER ("Foraminifera of the Upper Oligocene Cojimar Formation of Cuba," *Mem. Soc. Cubana Hist. Nat.*, 1940, **14**, No. 1, 19–35; No. 2, 113–32, pls. 17, 18; No. 3, 277–304, pls. 51–3). The Cojimar formation is a soft, light grey to cream-coloured marl which hardens on exposure and can be studied at numerous localities between Habana and Matanzas. Its fauna includes abundant echinoid spines, bryozoans, barnacles, mollusca, and foraminifera, and is very similar to that now living in the deep water off the coast of Cuba. The foraminiferal fauna comprises 203 species and varieties of 85 genera pertaining to 22 families. A few additional species cannot at present be identified owing to their rarity or unsatisfactory preservation. Many of the species can be compared with counterparts recently discovered by the "Atlantis" off the Cuban coast-line, but one camerinid *Operculinoides cojimarensis* has no living counterpart in the recent fauna of the region. A single specimen of *Miogypsina* sp. has been reported. Well-preserved specimens of *Globotruncana arca*, a distinctively Upper Cretaceous species were found in nine well-separated localities and cannot be summarily dismissed as derived Cretaceous fossils, as the Cojimar marl is a deep-water deposit accumulated well beyond the influence of currents or deposition from the erosion of coastal Cretaceous strata. All the evidence leads to the conclusion that the marl was deposited at a depth of not less than 200 fathoms. Thirty-one species and varieties are described as new. A. E.

Permian of New South Wales.—IRENE CRESPIN and W. J. PARR ("Arenaceous Foraminifera from the Permian Rocks of New South Wales," *Jour. and Proc. Roy. Soc., N.S.W.*, 1941, **74**, 300–11, pls. 12–3). Little research on the Permian Foraminifera of Australia has been done of recent years, but extensive collections of material collected by companies in search of oil have been investigated by the Commonwealth Palæontological Laboratory at Canberra. As a result this paper contains the description of one new genus *Digitina* (type *D. recurvata*), three new species: *Ammodiscus multicinctus*, *Ammobaculites woolnoughi*, and *Trochammina pulvillus*, and also records one species of *Hyperamminoides*, probably referable to *H. proteus* (Cushman and Waters) an American form, and a cosmopolitan species *Textularia eximia* (Eichwald). The new genus *Digitina* recalls Brady's genus *Climacammina* but does not develop the terminal uniserial chambers or a cribrate aperture. The wall is coarsely arenaceous and the cement apparently siliceous, though it may have been originally calcareous. Its systematic position is obscure but is tentatively assigned to the *Verneuilinidæ*, a family which has not been recorded earlier than the Jurassic. A. E.

Florida Wells.—W. STORRS COLE, ("Stratigraphic and Palæontologic Studies of Wells in Florida," *State of Florida Geol. Bull.*, No. **19**, 1941, i–vi, 1–53, pls. 1–18, tables and text-figs. 1–4). An exhaustive study of cores taken from two well-borings. The first in Central Florida was a water-well penetrating to a depth of 550 feet and reaching to the Middle Eocene. The second boring near the southern tip of Florida is the deepest so far made in that State. It was driven to a depth of 10,006 feet before being abandoned in what the author regards as Upper Cretaceous strata, though this is nearly 2000 feet below what some authorities have regarded as the top of the Lower Cretaceous beds. It is stated that though satisfactory correlations can be made in the younger formations encountered in these two wells, definite statements concerning the formations below the Middle Eocene must await the analysis of many more wells owing to the lack of fossils in the older formations. Thirty-four species and varieties of Foraminifera are described and well illustrated. They are mainly of the larger forms and include two new genera: *Pseudochrysalidina* (type *P. floridana*), which resembles *Chrysalidina* d'Orbigny, 1839, in form but

has a calcareous instead of an arenaceous test-wall; and *Discorinopsis* (type *D. gunteri*), which appears to resemble *Discorbis*, but has the umbilical area filled with spongy material containing a series of openings. Both the new genera were found in the Middle Eocene strata only. Other new species are: *Textularia coryensis*, *Valvulina floridana*, *Lituonella floridana*, *Coskinolina floridana*, *Spirolina coryensis*, and *Borelis gunteri*, and variety *floridana*. The new species are from the Middle Eocene except *Borelis*, which occurs only in Lower Eocene strata. A. E.

Upper Cretaceous of Colombia.—J. A. CUSHMAN and K. D. HEDBERG ("Upper Cretaceous Foraminifera from Santander del Norte, Colombia, S.A.," *Cont. Cush. Lab. For. Res.*, 1941, **17**, No. 232, 79–100, pls. 21–3). Upper cretaceous shales of great thickness in the north-eastern part of Colombia can be zoned readily on the basis of their Foraminifera. Three zones are described which beginning from the bottom are named after their dominant species: *Pullenia cretacea*, *Siphogenerinoides bramlettei*, and *Ammobaculites colombianus*, the last being notable for its arenaceous species, while in the two lower zones calcareous species predominate. The majority of the Colombian species are also found in the Upper Cretaceous of the North American Gulf Coast. Sixty-seven species in all are described and excellently figured. They include six new species and two new varieties. A. E.

Another New Genus.—J. A. CUSHMAN and PEDRO J. BERMUDEZ ("*Cuneolinella*, a New Genus from the Miocene," *Cont. Cush. Lab. For. Res.*, 1941, **17**, No. 233, 101–2, pl. 24, figs. 1–9). *Cuneolinella* is related to *Textulariella* and *Cuneolina*, both of which genera attain a large size in the Miocene of the West Indian region and are still living in the same area. It differs from the former in the strong compression of the test and its complex aperture, and from the latter in its aperture which, in the adult, consists of a series of elongate openings at the base of the inner margin of the last formed chamber. It attains to a diameter of 7–8 mm. The type *C. lewisi* is from Santiago Province, Dominica. There is also a variety —*intermedia*—differing in its less strongly compressed test, less produced peripheral angles, and fewer apertural openings. A. E.

Structure of Laticarinina.—J. A. CUSHMAN and RUTH TODD ("The Structure and Development of *Laticarinina pauperata* (Parker and Jones)," *Cont. Cush. Lab. For. Res.*, 1941, **17**, No. 234, 103–5, pl. 24, figs. 10–12). Describes in detail the minute structure of the test which seems to show very definitely that the genus is closely allied to and derived from *Cibicides*. Galloway and Wissler, who named the genus, described it as "test-free or attached to plants," and the authors would like to know more of its life habits, whether attached or free, and whether pelagic. But as it is a deep-water form and found far away from land, there is no possibility of attachment to weed, floating or otherwise. Its size and weight preclude the pelagic habit. By all analogy the broad keel is a device for keeping the organism in the surface layer of ooze. A. E.

More about Laticarinina.—J. A. CUSHMAN and RUTH TODD ("The Recent and Fossil Species of *Laticarinina*," *Cont. Cush. Lab. For. Res.*, 1942, **18**, No. 236, 14–20, pl. 4). The authors have made an exhaustive study of the literature and all available material, fossil and recent, in connection with this genus. The results seem to indicate that it occurs as early as the Cretaceous, but specimens with true generic characters have not been found by the authors earlier than the Miocene. The type species *pauperata* has a range from 56° N. to 71° S. and is found in practically all seas, though the Mediterranean does not figure in the details given. As a fossil *L. pauperata* has been recorded by various authors as far back as the Upper Cretaceous of New Zealand, but the authors state that they have not found typical *L. pauperata* in any fossil material they have examined. They also regard its wide

distribution as probable evidence of its attachment to floating seaweed, but except in the neighbourhood of land and in the converging accumulations of the Sargasso Sea, floating seaweed is of rare occurrence. There are two new species: *crassicarinata* from the Philippines and *bulbrooki* from the Miocene of Trinidad. The other known species of the genus are also described and figured. A. E.

Eocene Foraminifera from Trinidad.—J. A. Cushman and H. H. Renz ("Eocene, Midway, Foraminifera from Soldado Rock, Trinidad," *Cont. Cush. Lab. For. Res.*, 1942, **18**, No. 235, 1–14, pls. 1–3). Soldado Rock, a small island in the Serpents Mouth between Trinidad and Venezuela, supplied a yellowish marl containing both Orbitoids and small Foraminifera suggesting shallow water to reefal depositional conditions. The Foraminifera have much resemblance to the Midway Eocene of Texas, and Alabama but also contains numerous new species and varieties. Seven new species and two new varieties are figured and described out of a total list of thirty-six. The plates are remarkably good. A. E.

Post-glacial Foraminifera.—W. A. Macfadyen ("A Post-glacial Microfauna from Swansea Docks," *Geol. Mag.*, 1942, **79**, No. 2, 133–46). Test bores carried down to a depth of over 60 feet below O.D. showed a series of alternating peat beds and silty clays. Pollen analysis gives the age as the middle and second part of the Boreal period, when a rapid marine transgression was taking place. Eleven samples were examined. These were all from peaty beds and silty clays which presumably represent the nearly fresh-water phases of the series. The thicker intervening clays, which probably represent the more marine phases, have not been investigated. The microfauna recorded is mainly Foraminifera and is unusually rich compared with those of fenland deposits round the coast of Britain. Most of the samples have clear indications of a more marine facies.

The material yielded 127 species of presumed indigenous Foraminifera which is practically the same number as the author had previously found in 61 varied Fenland samples. There were also 15 varieties of other micro-fossils and 8 chalk derivatives. The Swansea fauna includes 42 species of indigenous Foraminifera not yet found by the author in British Fenland deposits; many of them are rare and in general are marine forms intolerant of brackish water. Of the common Fenland Foraminifera, 51 out of a total of 54 species were found. The Swansea clays thus include faunas representative of salinity ranging from marine to nearly fresh water, and the provisional conclusion reached is that the samples investigated were laid down under Fenland conditions, but that the open sea had comparatively ready, though somewhat filtered, access. In this connection the absence of *Elphidium crispum* (Linné) which is intolerant of brackish water is noteworthy.

There is one new name, *Bolivina britannica* for *Textularia variabilis* var. *lævigata*, Williamson *non* d'Orbigny. As this curious species is evidently confined to British seas the new specific name is very well chosen. A. E.

Report on Foraminiferal Soundings and Dredgings of the F.I.S. "Endeavour" along the Continental Shelf of the South-east Coast of Australia.—Frederick Chapman, A.L.S., Hon. F.R.M.S. (*Trans. Royal Society of South Australia*, 1941, **65** (2), 145–211, pls. 7–9). It is nearer sixty than fifty years since my old friend and correspondent Frederick Chapman published his first paper on Foraminifera. Since then they have been issued in a steady stream, and now, at an age when most men are content to rest on their oars, he not only publishes this noteworthy report but forecasts another on similar material. As he wrote to me recently his "enthusiasm for the forams is unabated." May it continue.

The material covered by the report was collected about 1912 by the late Captain

Dannevig in the F.I.S. *Endeavour* and was obtained from nine stations at the eastern end of the Bass Strait, three being on the Continental Shelf and the others on the Continental Slope. Their depths range between anchor mud and through shallow water (65 fathoms) down to 505 fathoms, and the majority of the samples come from below the mud line and consist of grey-green muds with a high organic content.

Apart from the Foraminifera which form the bulk of the report, some space is devoted to Mollusca, Polyzoa, and Ostracoda. The Polyzoa found were of thirteen species of which two are new to science (*Retepora babelensis* and *Mecynœcia* (*Entalophora*) *dannevigi*. No less than sixty-three species of Ostracoda are dealt with of which four species are new to science: *Cythere acerosella*, *C. postcaudispinosa*, *Cytheropteron hedleyi*, and *Cytherelloidea auris*. There is also one new name—*Pontocypris bradyi* (for *P. faba* G. S. Brady, 1878, *non* Reuss, 1855)—and a new variety *tenuis* of *Cythere obtusalata* G. S. Brady, 1880.

The Foraminifera are more numerous, 203 species in all being described. These include six new species: *Planularia australis*, *Bulimina notovata*, *Parafrondicularia helenœ* (named after Chapman's wife, who died recently and who, for nearly fifty years, had been his constant and devoted helper in his work on Foraminifera), *Notorotalia decurrens*, *Chilostomella cushmani*, and *Sigmoilina latissima*. There are also two new varieties: *Lagenonodosaria scalaris* var.n. *seminuda* and *Bolivinita quadrilatera* var.n. *tortilis*.

Chapman has already published two papers on *Endeavour* material in 1912 and 1915, and it is a pity that pressure of Official work delayed further research until now, for in the interim more than twenty of the species in this report have been described by various authors (including Chapman himself or with collaborators) from other localities.

The Foraminifera, as a whole, do not call for much comment. The majority are species which might be expected to occur at similar depths in most parts of the world. Based on a theory (partly confirmed by observation) that the area is a meeting point of two currents, a warm or Notonectian, flowing southwards from the Philippines and Torres Straits, and a cold, flowing northwards from the Antarctic, Chapman has attempted separate lists of species of presumed Notonectian and Antarctic origin. He is inclined to stress the point that these species, all from three of the deeper stations in 470–505 fathoms, are "strangers, brought from vast distances most likely through the agency of persistent currents."

But I fear that the mystery of the distribution of Foraminifera is not so easily solved. There are many known instances in which a particular species is found only in localities separated by thousands of miles. Indeed, there is one in this report: *Hyperammina novaezealandiœ*, discovered first off the North Cape, New Zealand, then found off the Falkland Islands, South Georgia, and to the west of Graham Land in the Antarctic, and now turning up again in the Bass Strait. It has thus circumnavigated the globe, by what means we cannot guess, as it is a large and bottom living form with practically no power of locomotion. As there is a continuous West Wind Drift right round the Southern Ocean it is not even possible to decide on the *locus* of origin, whether in New Zealand or in the Falklands, though the occurrence of the species on the eastern side of Graham Land projecting northward from the Antarctic continent, and the fact that it was not found in the Weddell Sea on the western side of Graham Land, seems to point to New Zealand or Australia as the original centre of distribution.

Apart from the pelagic Foraminifera, which of course are subject to current action, and some species of which figure in both of Chapman's lists, the position at present is that currents cannot be proved to influence the distribution of benthic species, and the problem remains unsolved. I have myself dealt with it at some

length in connection with the *Discovery* Antarctic Report (*Discovery Reports*, 1934, **10**, 19–21).

Chapman's lists do not, in my opinion, strengthen the "current" theory. Certainly there are a few species in each list which point definitely to a warm or cold water origin, as the case may be, but I should regard the majority as species of wide or even world-wide distribution at similar depths and temperatures. After all, it is temperature which regulates the distribution of the Foraminifera more than any other factor. Above the mud line, bottom temperatures are not widely different from that of the surface water, but below that line there is a steady drop with increasing depth until we reach a general minimum of 35°–36° F. in very deep water. Hence the distinctive regional faunas of shallow water, but while the benthic species of warm areas are isolated by the drop in temperature, those in Arctic or Antarctic shallow waters have no such restriction but can migrate freely into the deep sea. There are some species which have a tolerance for change of temperature and so have attained universal distribution. By what means is a still unsolved problem.

The temperatures of the *Endeavour* stations are not given but they are probably near the general mean for their depth, approximately 500 fathoms. The general mean for all oceans at this depth according to Murray is 40·1° F. as against 60·7° F. at 100 fathoms, the mud line. It is rather surprising that Chapman's lists contain a relatively small number of arenaceous species, which are usually a dominant feature at 500 fathoms.

Evidence of considerable current activity with fairly rapid changes of temperature are noticed in the sedimentation of plankton, an invariable feature where warm and cold currents meet. This is regarded by Chapman as evidence of good fishing grounds in the area. It may well be so, but, as Captain Dannevig was a recognized Fisheries expert, one would have expected him to report accordingly and that a fishing industry would have become established in the intervening years.

Further definite evidence on this junction of cold and warm water is produced in the interesting fact that Chapman found abundant rhombohedral crystals of calcite in the finer washings. Carbonate of lime in solution is a normal constituent of sea water, with a higher saturation point in warm water than in cold. A supersaturated warm water mixing with a colder water must part with its excess of salt to the saturation point of the lower temperature, and releases the excess in a crystalline form. Chapman had already found similar crystals in a Lower Miocene marl from Gippsland, but they were of larger size than those from the recent deposits, averaging 0·04 mm. in diameter as against 0·06 mm. in the recent material. He comments on the paucity of records of free calcite in recent sedimentary deposits, but this may be accounted for by their minute size which would allow them to pass through all but the finest sieves. Moreover, the people who work on marine deposits are, as a rule, solely interested in their organic contents and take little notice of mineral constituents. On the other hand, mineralogists have little interest in marine deposits, and between the two the presence or absence of calcite crystals goes unnoticed. Probably careful observations would lead to their discovery in many localities where currents impinge.

On one point only am I critical. Chapman wrote to me recently that he felt "like a watch-dog regarding those who prowl round seeking to establish new genera." There are no new genera here, but, following the American school, he uses the long disused genera *Lenticulina* Lamarck 1804, *Planularia* Defrance 1824, *Saracenaria* Defrance 1824, and *Astacolus* Montfort 1808, for various forms which the older school of rhizopodists (to which I think Chapman really belongs) referred to *Cristellaria* Lamarck 1812. This group of Foraminifera suffers from variation to such an extraordinary extent that no matter which of the many generic names

which have been proposed are accepted, there will always be found specimens intermediate between any two. The logical deduction would be to form additional new genera for these intermediate forms, but even Dr. J. A. Cushman, the leading American authority, admits that " no good purpose can be served by the use of too many names," and, while selecting six genera from the mob admits the difficulty of dealing with intermediate forms. Then why abandon the long-accepted *Cristellaria* as an " umbrella "-genus covering the group ?

Again Chapman has revived the stillborn genus *Lagenonodosaria* Silvestri 1900 for *Nodosaria* (*Nautilus*) *scalaris* Batsch 1791. I wonder why ? *N. scalaris* is not only one of the oldest but also a very widely distributed and variable species, and must have figured under that name in a multitude of papers. So far as my memory serves (I am unable to check my memory by the Zoological Index) nobody has ever used Silvestri's name, nor does it seem called for. Even in its most marked variations *N. scalaris* remains *Nodosaria,* and I think Chapman's " watchdog " attitude might well have been exercised here. A. E.

Arthropoda.

Arachnida.

Halacarids from the Red Sea and from Terra del Fuego.—ANDRÉ (" Description de six Halacariens de la Mer Rouge," *Bull. Mus. Hist. nat. Paris* (2), 1938, **10,** 57–63, 166–72, 6 text-figs ; " Descriptions de Halacariens de la Terre de Feu," *ibid.* (2), 1938, **10,** 271–8, 385–90, 6 text-figs.). On the ground that Trouessart had either failed to give figures or to describe satisfactorily the following : *Halacarus rostratus, H. parallelus, Copidognathus bavayi var. corallorum, C. gibbus var. cataphracta, Agauopsis exornata, Ischyrognathus coutieri,* author has described them anew and furnished detailed figures. BM/HNDH

Acarid Ontogenic Terminology.—GRANDJEAU (" Sur l'ontogenie des Acariens," *C.R. Acad. Sci. Paris,* 1938, **206,** 146–50). Author proposes new terms, e.g. " *calyptostase* " and the rejection of terms such as " *nymphochrysalis* " and " *teleiochrysalis* " with a discussion as to their applicability to acarine ontogeny. BM/HNDH

The Water Mite Genus Tyrrellia.—MARSHALL (" The Water Mite Genus Tyrrellia," *Trans. Wis. Acad. Sc.,* 1940, **32,** 383–9, 2 pls.). From material now available, author has been able to amplify the generic description of *Tyrrellia* which genus up to the present has not been found outside of North America. The description of the type has been revised and includes that of the male. *Tyrrellia circularis monensis,* n.var. was found in a warm spring (32·8° C.) on Paoha Island in Mona Lake, California. BM/HNDH

Wisconsin Hydracarina.—MARSHALL (" Preliminary List of the Hydracarina of Wisconsin," Part VI., *Trans. Wis. Acad. Sc.,* 1940, **32,** 135–65, 6 pls.). Continuing her investigations, author brings the Wisconsin list up to 30 genera containing 126 species and varieties and expects with further work to increase this number. Present list contains the following new species, viz. *Midea expansa, Mideopsis americanus, Arrenurus muttkowskii, A. wardi. A. megalurus intermedius* is a new variety. *Geayia ovata* (Wolc.), male, is here described. Males and nymphs formerly described as such, now appear to be referable to *Krendowskia similis* of which the female is now described. Original description of *Albia cærulea* was based on the study of two individuals then thought to be males but now recognized as nymphs. Male was later described and now female is dealt with. Material from Wisconsin, Illinois, and Indiana previously recorded as *Arrenurus parallelatus* is now referred to *A. wardi.* BM/HNDH

Cytology.

The Cytology of Bacteria, III. The Nuclear Apparatus as shown by Feulgen's "Nuclealfärbung" Reaction.—M. M. MOORE (*Proc. Roy. Irish Acad.*, 1941, **47**, Sect. B, 21). Critical review of previous work on presence of a nucleus in bacteria with earlier staining methods and with the Feulgen method. Feulgen-positive granules divide before division, and in spore formation migrate into the future spore. The granule in the resting spore divides when the spore germinates. J. A. M.

A Study of the Nuclear Apparatus of Bacteria.—C. F. ROBINOW (*Proc. Roy. Soc. B.*, 1942, **130**, 299). Feulgen, Giemsa, and iron alum hæmatoxylin were used after osmic acid vapour fixation. The nuclear apparatus of spores of sporing ærobes is a Feulgen-positive dumbbell-shaped element attached to a Feulgen-negative rod. On germination the dumbbell body enters the rod and becomes invisible. When it reappears it has divided into two dumbbell-shaped elements. Division of these chromatinic elements precedes division of the bacterial cell. Each vegetative cell usually contains a pair of dumbbells.

The author considers the dumbbell-shaped bodies as comparable to the chromosomes of plant and animal cells. J. A. M.

Heterochromatin in Triton.—H. G. CALLAN (*Proc. Roy. Soc. B.*, 1942, **130,** 324). When subjected to low temperature certain segments of the chromosomes of *Tritom vulgaris*, *T. palmatus*, and *T. cristatus* are heterochromatic. At mitosis these segments are undercharged and at meiosis uncharged with nucleic acid.

These segments show the same type of allocyclic behaviour as do similar segments in *Paris*, *Trillium*, and *Fritillaria*. They form Feulgen-positive chromocentres in all diffuse nuclei except pachytene, which is diffuse in *Triton.*

The availability of nucleic acid at the stage when the chromosomes normally spiralize is now shown to be a condition of that spiralization. The diffuse pachytene without chromocentres is followed by meiosis with unspiralized heterochromatin : the diffuse resting nucleus with chromocentres is followed by mitosis with spiralized heterochromatin.

With certain exceptions heterochromatin seems to be confined to those parts of chromosomes where chiasmata and crossing-over rarely occur. (Author's abstract.)

Tissue Culture and Serum Reaction with the Elementary Body Suspensions (E.B.S.) of Influenza Virus.—M. TSURUMI, K. OGASAWARA, T. KATO, and H. ABE (*Kitasato Arch. of Exp. Med.*, 1941, **18,** 52). Survival and proliferation of influenza virus in chick embryo—Tyrode emulsions. Passage was repeated every 48 hours for 18 generations. Elementary bodies were demonstrated in certain cells of tissue culture material. Agglutination of E.B. with rabbit-anti E.B.S. immune-serum was obtained. J. A. M.

Intoxication by Manganese in Well Water.—R. KAWAMURA, H. IKUTA, S. FUKUZUMI, R. YAMADA, and S. TSUBAKI, T. KUDAMA, and S. KURATA (*Kitasato Arch. Exp. Med.,* 1941, **18,** 145, 1 coloured plate). Old dry cells buried around the well supplying five families was source of contaminating manganese. P.M. of one fatal case showed degenerative changes in nerve cells with brown pigmentation. Manganese was found in the viscera. J. A. M.

The Development of the Megakaryocyte in Adult Mice.—J. S. POTTER and E. N. WARD (*Anat. Rec.*, 1940, **77,** 77). The megakaryocyte arises from the common mother cell of all blood cells, the hæmocytoblast, by nuclear enlargement following incomplete mitosis and increase of cytoplasm. They are incapable of self-perpetuation. Function remains ill-defined. J. A. M.

NOTICES OF NEW BOOKS.

Instructions for Collectors: No. 9A. Invertebrate Animals other than Insects.—By H. A. BAYLIS and C. C. A. MONRO. 1941. vi+74 pp., 2 text-figs., 12 plates. Price 1*s*. net.

No. 4A. Insects.—By JOHN SMART. 1940. vi+164 pp., 42 text-figs. British Museum (Natural History), S.W.7. Price 1*s*. 6*d*. net.

Mosquitoes of the Ethiopian Region. III. Culicine Adults and Pupae.—By F. W. EDWARDS. 1941. viii+499 pp., 184 text-figs., 4 coloured plates. British Museum (Natural History), S.W.7.

Animal Fibres of Industrial Importance: their Origin and Identification.—By A. B. WILDMAN. 1940. 28 pp., 5 text-figs., 23 plates. Wool Industries Research Association, Torridon, Headingley, Leeds 6.

Handbook of Chemical Microscopy.—By E. M. CHAMOT and C. W. MASON. Vol. II, Second Edition. 1940. xii+438 pp., 233 text-figs. Chapman & Hall, Ltd., 11 Henrietta Street, W.C.2. Price 30*s*. net.

Cytology and Cell Physiology.—Edited by GEOFFREY BOURNE. 1942. x+296 pp., 32 text-figs., 5 plates. Oxford University Press, Amen House, Warwick Square, E.C.4. Price 20*s*. net.

Insect Pests in Stored Products.—By H. HAYHURST. Second Edition. 1942. xii+108 pp., 54 plates containing 143 photographs. Chapman & Hall, Ltd., 11 Henrietta Street, W.C.2. Price 15*s*. net.

A Text-Book of Bacteriology.—By R. W. FAIRBROTHER. Fourth Edition. 1942. x+463 pp., 12 text-figs., 6 plates, 34 tables. Wm. Heinemann, 99 Great Russell Street, W.C.1. Price 17*s*. 6*d*. net.

The Handling of Chromosomes.—By C. D. DARLINGTON and L. F. LA COUR. 1942. 165 pp., 7 text-figs., 16 plates, 9 tables. George Allen & Unwin, Ltd., 40 Museum Street, W.C.1. Price 8*s*. 6*d*. net.

This quite unusual book deals with a subject that has an extensive literature and yet it provides a mine of information not to be found elsewhere. From the microscopist's point of view it provides much information on methods of using the instruments that are of value, although some of the suggestions are not exactly orthodox. Thus the box described for taking photo-micrographs, although there is some objection to the use of this term, is quite simple and practical. The use of the projected image is also advised for drawing purposes to which no objection can be offered. In fact the use of screen projection for demonstration purposes is one of the most useful portions of the book. It can be adapted for so many purposes that it should appeal to teachers and to workers, but whether a magnification reaching $\times 5000$ is desirable is questionable, although this seems to refer to class projection. The photo-reproductions are excellent and provide large-scale reproductions which are unrivalled. There is much technical information that the title of the book suggests. It will be a valuable guide to all workers on the subject.

J. E. B.

Carnegie Institute of Washington Publication No. 537.—Scientific Results of Cruise VII of the *Carnegie* during 1928–1929 under the command of Captain J. P. Ault. Biology—II: The oceanic tintinnoina of the plankton gathered during the last cruise of the *Carnegie*. By A. S. Campbell. 1942. vi+163 pp., 1 plate, 128 text-figs. Carnegie Institution of Washington. Price $1.50 in paper cover, $2.50 in cloth binding, post free.

There are discussed in this report 13 families, 44 genera, and 311 species (of which 307 were found in this material). There are three new subfamilies, two new genera, fourteen new species, one new variety, and one new name. The classification follows the thesis that the lorica affords a satisfactory means of arrangement, since this organ is the result of the internal organization of the animal.

The expedition covered a total distance of 44,877 nautical miles, the larger part of which was in the Pacific, the shorter part in the North and Tropical Atlantic. There were 160 stations. Collections were made, with rare exceptions, at the surface, at 50, and at 100 metres. Of 648 phials examined, 272 were from net samples and 376 from pump samples.

More complete and exact data than those reported by any earlier expedition are recorded for each species. Records of temperature, density, salinity, and pH are given for both Atlantic and Pacific samples for each of the 307 species in pump and net samples separately.

The limits of geographical distribution are given on the basis of the natural areas of distribution of Schott. Four general faunal regions are recognized.

Quantitatively significant results are recorded for every species, pump and net samples being segregated in the summaries.

The Journal of the Biological Photographic Association.—Vol. 10, No. 4. June, 1942. Biological Photographic Association, 558 Elizabeth Street, Waukesha, Wisconsin, U.S.A. Annual Subscription $3.00. Single copies 75 cents.

The purpose of this periodical is the advancement of photography in relation to the biological sciences and it must be admitted that we have no periodical in this country with similar aims. Its opening paper on " The Structural Variations of Motor Nerve Plates " is of interest and the illustrations are exceptionally good reproductions from Kodachrome plates. Clear instructions for the preparation of the material are given in the text together with the necessary photographic technique. It is clear that in this case photomicrographs in colour are a great advantage. A paper follows on the determination of exposure in photomicrographs by means of a Weston photoelectric cell. This is a more debateable subject, few workers with experience would be inclined to follow in all details. Some other papers follow which are more discursive, but they all have a definite purpose. It must be admitted that this very well-produced journal should have a good future, providing that subjects of sufficient interest can be found to maintain its high standard.

J. E. B.

PROCEEDINGS OF THE SOCIETY.

The Council orders for the information of the Fellows the publication of the following summary of the Society's Proceedings since the last report.

New Fellows.—The following have been elected Ordinary Fellows of the Society :—

C. F. Bause.	York.
J. R. Carter.	Bradford.
R. H. Chanter.	Leytonstone.
S. E. Eglinton.	Carluke.
G. H. Giles.	London.
F. J. Howe.	Rugby.
S. D. Mistry.	Bombay.
H. E. Moore.	Yukon.
J. H. Murgatroyd.	Stockport.
F. M. Nicholson.	Chicago.
J. R. Pownall.	Southport.
H. R. Priestley.	Stockton-on-Tees.

Deaths.—The Council regrets to report the death of the following Fellows :

J. J. R. Armitage.	Elected	1926.
B. L. Bhatia.	,,	1919.
C. T. Owen.	,,	1923.
A. W. Ryland.	,,	1917.
A. D. G. Shelley.	,,	1885.
Mansell J. Swift.	,,	1906.
O. Whittaker.	,,	1898.
P. P. Wilding.	,,	1910.
C. A. Woutersz.	,,	1936.

Donations.—The following donations have been received and duly acknowledged on behalf of the Fellows :—

F. D. Armitage, F.R.M.S.—

24 Reference slides of paper-making fibres.

D. J. Scourfield, *I.S.O.*, F.R.M.S.—

"A Key to the British Specie of Freshwater Cladocera." By D. J. Scourfield and J. P. Harding.

Clarendon Press—

"Cytology and Cell Physiology." Edited by Geoffrey Bourne.

Chapman and Hall, Ltd.—

"Insect Pests in Stored Products." By H. Hayhurst. Second Edition.

George, Allen and Unwin—

"The Handling of Chromosomes." By C. D. Darlington and L. F. La Cour.

Exors. of the late Prof. Gandolfi Hornyold, F.R.M.S.—

A French monocular microscope with accessories. By Stiassnie. Paris.
A small travelling microscope with accessories. By Zeiss.
A micro-polarizing equipment. By Zeiss.

Wm. Heinemann—

"A Text-Book of Bacteriology." By R. W. Fairbrother. Fourth Edition. 1922.

Carnegie Institution of Washington—

Publication No. 537. "The Oceanic Tintinnoina of the Plankton gathered during the last cruise of the *Carnegie*." By A. S. Campbell.

Evan G. MacLeod, F.R.M.S.—

Five guineas. (£5 5*s.* 0*d.*)

Grant in aid of publication—

Two hundred pounds. (£200)

Papers.—The following papers submitted have been considered and approved by the Council for publication in the Society's *Journal* :—

H. Emmett—

"Cinemicrography in Scientific Research."

E. E. Jelley, D.Sc., F.R.M.S.—

"A Review of Crystallographic Microscopy."

E. E. Trueman—

"The Structure and Deposition of the Shell of *Tellina tenuis*."

J. R. Baker—

"Tube-length in Photomicrography."

P. N. Bhaduri, F.R.M.S.—

"Chromosome-Nucleolus relationship and its bearing on Cytogenetical Interpretation."

J. M. Watson—

"The Ripening of Ehrlich's Haemotoxylin."

T. E. Wallis, F.R.M.S. and J. W. Fairbairn—

"Determination of Nux Vomica in the Form of Powder."

Treasurer's Report.—The following report and accounts in respect of the years 1940 and 1941 have been received and approved by the Council:—

TREASURER'S REPORT AND BALANCE SHEETS FOR THE YEARS ENDED 31ST DECEMBER, 1940 AND 1941.

In presenting the audited accounts and Balance Sheets for the past two years I have to report that the audit has been effected under exceptionally difficult and hazardous conditions during which the premises of the auditors were completely destroyed by enemy action, involving the total loss of the Society's books of accounts and records covering a period of several years.

In these circumstances the reconstruction of the Society's accounts has proved a protracted and arduous task for both the Secretary and the Auditors, and I feel sure I am expressing the wishes of all the Fellows in placing on record our sense of appreciation and thanks for their outstanding services to the Society in this important matter.

The noticeable drop in revenue was perhaps to be expected as a result of war conditions and their effect upon subscriptions, both in respect of the Fellowship and of the Society's Journal. With regard to the Journal the decrease in revenue through the temporary loss of so large a part of its world-wide circulation, coupled with the emergency restrictions and increasingly heavy costs of printing and publication, would have proved a most serious matter for the Society's resources had it not been for the generous financial aid received for which the grateful thanks of the Council have been duly conveyed to the donors.

With regard to the Investments, the Society has been required to surrender to H.M. Treasury its holding of £915 11*s*. 4*d*., India 3 p.c. Stock, and the Council has directed that the proceeds therefrom be invested in 3 p.c. Defence Bonds and 3 p.c. Savings Bonds.

On the recommendation of the Auditors the Council has directed that of the reserve held in 3 p.c. Defence Bonds a sum be specifically earmarked against the amount at present at credit of Superannuation Reserve and the amount to be provided in respect of the year 1942.

A sum of £100 has been reserved as a necessary post-war rehabilitation fund in respect of the Society's furniture and equipment.

In submitting these accounts I take this opportunity to acknowledge with deep appreciation the loyal support of the Fellows without which the uninterrupted maintenance of the Society's essential activities under prevailing circumstances would prove most difficult to achieve.

(Signed) C. F. HILL,
Honorary Treasurer.

Dr. INCOME AND EXPENDITURE ACCOUNT FOR

1939 £ s. d.	Expenditure.	£ s. d.	£ s. d.
95 11 2	To balance, being Excess of Expenditure over Income at 31st December, 1939		247 14 11
165 15 8	" Rent, Telephone and Insurance		166 0 11
354 0 0	" Salaries, Reporting, etc.		350 0 0
	" Sundry Expenses—		
	Library Books and Binding	6 16 3	
	Stationery, Printing, Postages and Sundry Expenses	36 18 9	
	Repairs and Renewals to Furniture, etc.	—	
	Refreshments at Meetings	—	
132 10 10			43 15 0
	" Journal—		
	Expenditure—		
	Printing	411 2 3	
	Editing and Abstracting	190 8 6	
	Illustrating	23 2 11	
	Postages and Addressing	22 10 1	
744 9 5			647 3 9
16 7 7	" Depreciation on Furniture		14 14 10
	" Superannuation Reserve—		
	Annual Instalment	100 0 0	
	Interest	18 15 4	
114 15 0			118 15 4
£1623 9 8			£1588 4 9

THE YEAR ENDED 31st DECEMBER, 1940. Cr.

1939. £ s. d.	Income.	£ s. d.	£ s. d.
	By Members' Subscriptions and Journal Sales	975 4 10	
	,, Members' Subscriptions for 1940 unpaid	86 2 6	
1154 11 10			1061 7 4
64 4 8	,, Journal Advertisements		50 11 9
—	,, Grant received towards expenses of publication of Journal		50 0 0
29 4 3	,, Donations and Sundry Receipts		44 19 1
	,, Interest on Investments and Deposit Account—Gross	148 16 2	
	Less: Income Tax deducted	30 16 6	
127 14 0			117 19 8
247 14 11	,, Balance, being Excess of Expenditure over Income at 31st December, 1940		263 6 11
£1623 9 8			£1588 4 9

BALANCE SHEET AS

Liabilities.	£	s.	d.	£	s.	d.	£	s.	d.
I. *Capital*—									
Being (*a*) Life Compounded Subscriptions from 1st January, 1877 to 31st December, 1939 .	2117	3	0						
Add: Received during 1940	—								
				2117	3	0			
(*b*) Quekett Memorial Fund				100	0	0			
(*c*) Mortimer Bequest				45	0	0			
(*d*) A. N. Disney Bequest				100	0	0			
(*e*) Amounts received in respect of Sales of Books and Catalogues from the Library (surplus to the Society's requirements), as at 31st December, 1939 . . .	317	8	10						
Add: Sales during 1940 .	1	1	6						
				318	10	4			
(*f*) Admission Fees for 1931 to 1939	449	8	0						
Add: Fees for 1940 . .	8	8	0						
				457	16	0			
				3138	9	4			
Less: Amount transferred to Superannuation Reserve in respect of the year ended 31st December, 1935				100	0	0			
							3038	9	4
II. *Sundry Creditors*—									
Subscriptions paid in advance				4	14	6			
Journal Subscriptions paid in advance				19	19	0			
On Account of Journal Printing, Rent, etc. . .				376	12	0			
Reserve for Expenses in connection with the Centenary Meeting				211	14	0			
							612	19	6
III. *Superannuation Reserve*									
Instalments and Interest 1935 to 1939 . . .				536	4	11			
Transfer from Income and Expenditure Account—									
Instalment for the year ended 31st December, 1940	100	0	0						
Interest at 3½% per annum. . .	18	15	4						
				118	15	4			
							655	0	3
							£4306	9	1

C. F. Hill, *Hon. Treasurer.*

Certified in terms of our Report

AT 31ST DECEMBER, 1940.

Assets.	£	s.	d.	£	s.	d.
I. *Furniture and Equipment*—						
As at 31st December, 1939	147	8	1			
Less : Depreciation at 10%	14	14	10			
				132	13	3
II. *Investments at Cost*, less amounts written off :—				2996	17	8
£400 London and North Eastern Railway Co. 3% Debenture Stock.						
£500 Nottingham Corporation 3% Irredeemable Debenture Stock.						
£915 11*s.* 4*d.* 3% India Stock, 1948.						
£150 Metropolitan Water Board, "B" Stock.						
£612 London Midland and Scottish Railway Co. 4% Preference Stock.						
£200 New South Wales, 5¼% Loan, 1947–57.						
£200 5% Conversion Loan, 1944–64.						
£100 3% Conversion Loan, 1948–53.						
£421 1*s.* 0*d.* 3½% War Stock (Registered).						
£400 3½% War Stock (Inscribed).						
£300 Birmingham Corporation 3¼% Redeemable Stock, 1957–62.						
Note.—The Market Value of the above Investments at 31st December, 1940, was approximately £3557 5*s.* 7*d.*						
III. *Sundry Debtors.*						
Subscriptions unpaid and amounts due in respect of Journal Sales, Advertisements, etc.				186	9	9
IV. *Cash at Bank and in Hand.*						
At Bank on Deposit Account	500	0	0			
At Bank on Current Account	227	1	6			
In Hand	—					
				727	1	6
V. *Income and Expenditure Account*—						
Balance, being Excess of Expenditure over Income, as per Account attached				263	6	11
				£4306	9	1

of 11th August, 1942.

(Signed) THOMSON McLINTOCK & CO.,
Chartered Accountants, Hon. Auditors.

Granite House, 101, *Cannon Street, London, E.C.*4.

Dr. INCOME AND EXPENDITURE ACCOUNT FOR

1940. £ s. d.	Expenditure.	£ s d.	£ s. d.
247 14 11	To Balance, being Excess of Expenditure over Income at 31st December, 1940		263 6 11
166 0 11	,, Rent, Telephone and Insurance		166 17 6
350 0 0	,, Salaries		350 0 0
	,, Sundry Expenses :—		
	Library Books and Binding	3 17 9	
	Stationery, Printing, Postages and Sundry Expenses	71 17 1	
	Repairs and Renewals to Furniture, etc.	4 13 1	
43 15 0			80 7 11
	,, Journal—		
	Expenditure :—		
	Printing	226 14 6	
	Editing and Abstracting	170 18 11	
	Illustrating	16 17 6	
	Postages and Addressing	9 16 1	
647 3 9			424 7 0
—	,, Reserve for Repairs to Instruments, etc.		100 0 0
14 14 10	,, Depreciation on Furniture		13 5 4
	,, Superannuation Reserve :—		
	Annual Instalment	100 0 0	
	Interest	22 18 6	
118 15 4			122 18 6
£1588 4 9			£1521 3 2

THE YEAR ENDED 31st DECEMBER, 1941. Cr.

INCOME.

1940.				£	s.	d.	£	s.	d.
			By Members' Subscriptions and Journal Sales	892	8	9			
			,, Members' Subscriptions for 1941 unpaid .	51	19	6			
1061	*7*	*4*					944	8	3
50	*11*	*9*	,, Journal Advertisements				37	4	6
50	*0*	*0*	,, Grant received towards expenses of publication of Journal				250	0	0
44	*19*	*1*	,, Donations and Sundry Receipts . . .					15	9
			,, Interest on Investments and Deposit Account —Gross	153	6	11			
			Less : Income Tax deducted . .	32	0	11			
117	*19*	*8*					121	6	0
263	*6*	*11*	,, Balance, being Excess of Expenditure over Income at 31st Decmber, 1941				167	8	8
£1588	*4*	*9*					£1521	3	2

BALANCE SHEET AS

LIABILITIES.	£	s.	d.	£	s.	d.	£	s.	d.
I. *Capital*—									
Being (*a*) Life Compounded Subscriptions from 1st January 1877 to 31st December, 1940 .	2117	3	0						
Add : Received during 1941	—								
				2117	3	0			
(*b*) Quekett Memorial Fund				100	0	0			
(*c*) Mortimer Bequest				45	0	0			
(*d*) A. N. Disney Bequest				100	0	0			
(*e*) Amounts received in respect of Sales of Books and Catalogues from the Library (surplus to the Society's requirements), as at 31st December, 1940 . . .	318	10	4						
Add : Sales during 1941 .	4	9	4						
				322	19	8			
(*f*) Admission Fees for 1931 to 1940	457	16	0						
Add : Fees for 1941. . . .	31	10	0						
				489	6	0			
				3174	8	8			
Less : Amount transferred to Superannuation Reserve in respect of the year ended 31st December, 1935 . . .				100	0	0			
							3074	8	8
II. *Sundry Creditors*—									
Subscriptions paid in advance.				7	18	0			
Journal Subscriptions paid in advance . . .				32	18	0			
On Account of Journal Printing, Rent, Repairs, etc.				293	16	11			
Reserve for Expenses in connection with the Centenary Meeting				211	14	0			
							546	6	11
III. *Superannuation Reserve*—									
Instalments and Interest, 1935 to 1940 . . .				655	0	3			
Transfer from Income and Expenditure Account— Instalment for the year ended 31st December, 1941	100	0	0						
Interest at 3½% per annum . . .	22	18	6						
				122	18	6			
							777	18	9
C. F. HILL, *Hon. Treasurer.*							£4398	14	4

London, 11*th August*, 1942. We have examined the Books and Accounts of the Royal Microscopical Society or, in the case of records destroyed by enemy action, statements reconstructed from the documents and data available to us, for the two years ended 31st December, 1940 and 31st December, 1941, respectively, and have prepared therefrom the Balance Sheets annexed hereto which in our opinion are properly drawn up so as to exhibit

AT 31st DECEMBER, 1941.

Assets.	£	s.	d.	£	s.	d.
I. *Furniture and Equipment*—						
As at 31st December, 1940	132	13	3			
Less : Depreciation at 10%	13	5	4			
				119	7	11
II. *Investments at Cost*, less amounts written off :— . . .				2565	10	3
£400 London and North Eastern Railway Co. 3% Debenture Stock.						
£500 Nottingham Corporation 3% Irredeemable Debenture Stock.						
£150 Metropolitan Water Board, "B" Stock.						
£612 London Midland and Scottish Railway Co. 4% Preference Stock.						
£200 New South Wales 5½% Loan, 1947–57.						
£200 5% Conversion Loan, 1944–64.						
£100 3% Conversion Loan, 1948–53.						
£421 1*s.* 0*d.* 3½% War Stock (Registered).						
£400 3½% War Stock (Inscribed).						
£300 Birmingham Corporation 3¼% Redeemable Stock, 1957–62.						
£400 3% Defence Bonds.						
Note.—The Market Value of the above investments at 31st December, 1941 was approximately £3,413 14*s.* 0*d.*						
III. *Sundry Debtors*—						
Subscriptions unpaid and amounts due in respect of Journal Sales, Advertisements, etc. less reserve .	101	6	3			
Proceeds of £915 11*s.* 4*d.* India 3% Stock (1948 or later) acquired by H.M. Treasury, for settlement 2nd March, 1942	831	7	5			
				932	13	8
IV. *Cash at Bank and in Hand*—						
At Bank on Deposit Account	500	0	0			
At Bank on Current Account	113	3	8			
In Hand		10	2			
				613	13	10
V. *Income and Expenditure Account*—						
Balance, being Excess of Expenditure over Income, as per Account attached				167	8	8
				£4398	14	4

a true and correct view of the state of the Society's affairs according to the best of our information and the explanations given to us, subject to it being noted that no account has been taken of the value of the Society's Library, Stock of Journals, and Collection of Instruments (valued for insurance together with the Furniture and Equipment at £7,000).

(Signed) THOMSON McLINTOCK & CO.,

Chartered Accountants, Hon. Auditors.

Granite House, 101, *Cannon Street*, *London*, *E.C.*4.

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INDEX OF AUTHORS